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THE ADRENAL LIPIDS IN PREGNANT RABBITS

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The adrenal glands are known to change size rapidly under a wide variety of conditions (1) and consequently provide an interesting organ for the comparison of chemical composition and physiological activity. It has long been known that the adrenal gland contains a relatively large amount of lipid and that the lipid fractions are present in unusual proportions. It resembles blood serum rather than other tissues in its lipid proportions, especially in its cholesterol-ester content. For this reason, theories have been proposed that the adrenals actually exert some control over the serum-lipid level; particularly, that the adrenals synthesize or store cholesterol for the maintenance of the blood-serum-cholesterol level. Although it is possible that the adrenal glands play a part in the regulation of the blood-serum level via their hormones, it is highly improbable that they could synthesize or store sufficient lipid to influence appreciably the serum-lipid level.

Except for histological studies, which are difficult to interpret, the relation of adrenal hypertrophy or atrophy to the lipid composition has not been extensively studied. In the adrenal hypertrophy, produced by insulin-hypoglycemic treatments, it was found that phospholipid, neutral fat and total lipid increased in absolute amounts while cholesterol esters and free cholesterol were unchanged (2). When calculated as a percentage of the moist weight, the phospholipid, free cholesterol and total lipid content remained constant while the ester cholesterol decreased and the neutral fat increased in the hypertrophying organ. Cholesterol feeding (3) caused hypertrophy of the adrenals and increase in the phospholipid, free and ester cholesterol, neutral fat and total lipid. Blumenfeld (4) observed that the absolute amount of total lipid in the adrenal glands of spayed rats was decreased in proportion to the degree of atrophy. In the

adrenal hypertrophy of pregnancy and parturition, Anderson and Sperry (5) observed that both the relative and absolute amounts of cholesterol esters, but not free cholesterol, were decreased as compared with those of adrenals in various phases of the oestrus cycle. In addition, the cholesterol esters were decreased in the adrenals of spayed rats. They concluded that cholesterol esters in the adrenal glands are not related to physiological states since spayed animals have decreased and pregnant and parturient rats have increased metabolic processes.

In the present study, we have examined the weights and lipid composition of the adrenals in control, pregnant and pituitary-treated rabbits.

METHODS. The methods for the analyses of the adrenal lipids were given in a previous publication (2). The data on the male control rabbits were also given (2). Young female rabbits (3–6 months) were used for the pregnancy and pituitary-injected series. The pituitary preparation was an acetone-insoluble, water-soluble fraction of horse pituitary the activity of which was determined by the production of ovulation. Protocols are given in another publication by Graubard and Pincus (6). The data herein reported were studied for their statistical validity by use of Fisher's "t" test.

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{N_1 + N_2}{N_1 N_2 (N_1 + N_2 - 2)}\left[Sx_1^2 - \frac{(Sx_1)^2}{N_1} + Sx_2^2 - \frac{(Sx_2)^2}{N_2}\right]}}$$

The probabilities attaching to the t's were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION. The data obtained are presented in tables 1 and 2. It is apparent that the pituitary preparation had no effect on the weight and lipid composition of the adrenals. These results are in agreement with those of Anderson and Sperry (5) that cholesterol and its esters do not vary significantly during various phases of the oestrus cycle.

During the first 6 days of gestation, in which there was no significant change in the weight of the adrenals, the lipid composition changed relatively little; only cholesterol esters and neutral fat were appreciably different. Although the percentage of cholesterol esters was decreased, the absolute amounts were not changed; consequently, the variation in the percentage of cholesterol esters must be attributed to the variations in adrenal weight. A real increase in both relative and absolute amounts of neutral fat occurred.

In the latter part of gestation there was marked increase in adrenal weights and proportionate changes in lipid composition. In the hypertrophied organs there was a significant decrease in phospholipid and cholesterol esters, no change in free cholesterol, and an increase in neutral fat and total lipid. However, the absolute amounts of the various lipid

fractions (calculated as milligrams per kilogram body weight) have increased in the pregnant series. Neutral fat increased relatively more than the other lipid fractions. These results are in contrast to those of Anderson and Sperry (5) who found a decrease in both absolute and relative amounts of cholesterol ester in pregnancy.

The indications are that the hypertrophying adrenal glands absorb neutral fat from the blood stream first and in greatest quantities. Cholesterol, either free or as the ester, must be absorbed also. The large amounts of neutral fat accumulated may be part of the mechanism for the synthesis of phospholipid by the growing cells in the process of hypertrophy. If it be assumed that hypertrophy of glands is caused by increased demand upon them and that the increased size is an indication of increased activity, the increase in the absolute amount of phospholipid in pregnant and insulin-treated rabbits may indicate a relationship between phospholipid content and physiological activity. At least, phospholipid appears to be related to the physiological activity of ovaries and muscles (8, 9).

The absolute amounts of cholesterol and its esters increase in the hypertrophy of pregnancy of rabbits but not of pregnant or parturient

TABLE 1

Lipid composition of adrenals in control, pituitary-treated and pregnant rabbits

		CONTROL	PITUITARY TREATED	PREGNANT		PROBABILITIES					
				1–6 days	19–28 days	Control vs. pituitary treated		Control vs. 1–6 day pregnant		Control vs. 19–28 day pregnant	
						t.	p.	t.	p.	t.	p.
Number of animals		20	21	16	9						
Adrenal, mgm. weight	M.*	213	261	281	594	1.65	>0.05	1.95	>0.05	9.96	<0.01
	S.D.	48	58	142	159						
Phospholipid, per cent	M.	3.57	3.57	3.66	2.94	0	>0.05	0.70	>0.05	4.23	<0.01
	S.D.	0.37	0.33	0.40	0.22						
Total cholesterol, per cent	M.	6.30	6.57	5.13	4.67	0.70	>0.05	3.04	<0.01	3.36	<0.01
	S.D.	1.42	1.03	0.66	0.35						
Free cholesterol, per cent	M.	0.86	0.88	0.86	0.96	1.20	>0.05	0.93	>0.05	1.75	>0.05
	S.D.	0.22	0.16	0.22	0.25						
Ester cholesterol, per cent	M.	5.50	5.69	4.27	3.71	0.59	>0.05	3.68	<0.01	4.30	<0.01
	S.D.	1.22	0.83	0.61	0.32						
Neutral fat, per cent	M.	5.82	5.12	8.48	12.54	0.93	>0.05	2.49	<0.05	4.29	<0.01
	S.D.	2.93	1.79	3.47	4.31						
Total lipid, per cent	M.	19.26	18.87	19.50	23.18	0.35	>0.05	0.19	>0.05	2.21	<0.05
	S.D.	4.46	2.44	2.86	4.29						

* M., mean; S.D., standard deviation.

rats (5) nor in insulin-treated rabbits (2). In the hypertrophy produced by cholesterol feeding of rabbits there is an accumulation of cholesterol esters (3). However, in the hypertrophy associated with infections there is a decrease in cholesterol esters (7). It would appear that the cholesterol content of the adrenals might be more closely related to the nutritional status of the animal than to the physiological status of the glands.

The adrenal glands appear to be unique and therefore of unusual interest in their ability to change size and lipid content rapidly under numerous physiological conditions. Most tissues, with the possible exception of the

TABLE 2

Analysis of lipid composition of adrenals as milligram per kilogram body weight

		CONTROL	PREGNANT		PROBABILITIES			
			1-6 days	19-28 days	Control vs. 1-6 day pregnant		Control vs. 19-28 day pregnant	
					t.	p.	t.	p.
Body weight, kgm.	M.	2.33	2.59	3.18				
Phospholipid	M.	3.52	3.75	5.46	0.53	>0.05	4.26	<0.01
	S.D.	1.21	1.39	0.92				
Total cholesterol	M.	5.88	5.42	8.67	0.59	>0.05	3.25	<0.01
	S.D.	2.28	2.36	1.77				
Free cholesterol	M.	0.75	0.94	1.82	1.38	>0.05	6.09	<0.01
	S.D.	0.30	0.53	0.66				
Ester cholesterol	M.	5.18	4.48	6.85	1.10	>0.05	2.36	<0.05
	S.D.	1.92	1.88	1.30				
Neutral fat	M.	5.69	9.49	23.77	2.13	<0.05	6.91	<0.01
	S.D.	3.81	6.77	10.42				
Total lipid	M.	18.46	21.29	43.67	0.91	>0.05	6.43	<0.01

liver, have a constant composition of phospholipid and cholesterol which does not change readily under a wide variety of conditions, including starvation. The adrenal gland should provide an interesting organ for studying chemical composition and physiological activity as measured by hormonal output.

SUMMARY

In the hypertrophied adrenal glands of pregnant rabbits there was an increase in the absolute amounts of phospholipid, free and ester cholesterol, neutral fat and total lipid.

The percentage content of phospholipid and cholesterol esters was decreased; free cholesterol and total lipid were not changed; and neutral fat was increased.

A gonadotropic hormone preparation of the pituitary had no significant effect on the adrenal lipids.

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QUANTITATIVE MEASUREMENT OF THE FIBRILLATION THRESHOLDS OF THE MAMMALIAN VENTRICLES WITH OBSERVATIONS ON THE EFFECT OF PROCAINE[1]

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The suspicion has existed for a long time that physiological, physical and chemical influences may create either a state of predisposition or of resistance to fibrillation. Various experimental methods have been designed to test and prove this idea. For these tests different species of animals have been used. Since fibrillation is permanent in the dog's heart, recourse has been taken to a study of apparently related ectopic ventricular rhythms or to comparison of crude fibrillation thresholds in series of treated and untreated animals. For example, Moisset de Espanès (1) found that stronger tetanizing currents were necessary to induce ventricular fibrillation in dogs after administration of 5 to 20 mgm. quinidine or 2 to 10 mgm. fagarina I per kilo. He also noted that the average time for development of spontaneous fibrillation was reduced after occlusion of the ramus descendens in animals previously treated with fagarina I. Thus, in one group of 25 normal dogs, spontaneous fibrillation occurred within 19 minutes in fifteen; in another group of 25 treated with quinidine, 20 fibrillated in less than 16 minutes, and in a third group of 25 treated with faragina I, only 9 fibrillated spontaneously in less than 15 minutes. Whether this means that quinidine acts unfavorably and faragina favorably during coronary occlusion, as this investigator believes, or whether the fibrillation time was fortuitous in these different groups may well be debated. The great variation in the fibrillation time after acute coronary occlusion is well known to experimenters. On the basis of fibrillation times, we could easily assemble three much larger groups from our experimental series of untreated dogs which would show even more significant variations.

Meek et al. (2) used identical dogs by studying the reactions to test doses of epinephrine which in the unanesthetized state only caused "slowing

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[2] Fellow of the Belgian-American Educational Foundation.

with or without escape of the A-V node, bundle or ventricle, but never tachycardia or fibrillation." They found, in 17 dogs, that a similar test dose caused essentially the same effect during light or deep anesthesia with ether or chloroform; but under light cyclopropane anesthesia, 11 of these dogs developed ventricular tachycardia lasting on an average 19 seconds, and one fibrillated. Under deep cyclopropane anesthesia, 16 developed tachycardia lasting 44.5 seconds (average) and one fibrillated. Aside from the two deaths due to fibrillation, the remainder of their proof requires the assumption that development of ectopic ventricular beats or tachycardia is necessarily linked with liability to fibrillate. Such a view would hold if the tachysystolic rather than circus movement concept of fibrillation is favored, which we are not ready to do.

Shen and Simon (3) found that 6 dogs succumbed to "chloroform-adrenalin" fibrillation, whereas of another series of 9 dogs protected by 3 to 5 mgm./kilo procaine hydrochloride, only one fibrillated. Similarly, Shen (4) showed that whereas 8 dogs died from "benzol-adrenalin" fibrillation, in another group of 7 dogs protected by 8 to 10 mgm./kilo procaine, fibrillation never developed. Smaller doses administered to another group of 5 dogs failed to protect. Burstein and Marangoni (5) reported that fibrillation was produced in 3 out of 5 dogs by administering epinephrine during cyclopropane anesthesia; but in 8 dogs previously treated with procaine, only one succumbed to fibrillation. A review of these and similar tests indicates the difficulty of obtaining decisive answers by the use of such bio-assay methods; so many fortuitous factors influence the induction of fibrillation by ischemia or drugs.

The cat has constituted a favored test preparation because the ventricles more generally recover from fibrillation within a minute or so, either spontaneously or by the aid of gentle massage. In this way, repeated thresholds can be obtained on the same animal, before and after various experimental measures. The criteria of changes in sensitivity to fibrillating agents are various and have not been too critically established. They may be grouped, and a few suggestions of their applicability examined.

A first method is based on determinations of the strength or duration (in seconds) of faradic or sine currents which are just sufficient to induce ventricular fibrillation.

Hoff and Nahum (6) applied 60 cycle alternating currents through the limbs of cats, keeping the strength constant but varying the duration. They report that A.C. shocks of shorter duration induce fibrillation following the use of epinephrine, but shocks of longer duration are required after subcutaneous administration of acetylcholine. Moisset de Espanès (1) supplemented his studies by determining the fibrillation threshold of a tetanizing current from an induction coil, and Van Dongen (7), if

we interpret his diction correctly, similarly induced ventricular fibrillation by tetanizing the auricles. We must seriously question the value of such thresholds on several grounds: 1. If, as our results and those of others indicate, a very brief unitary stimulus applied during the vulnerable period of a single systole can induce fibrillation (8, 9), the use of currents extending over numerous beats cannot be expected to be very informative. 2. Employment of prolonged oscillating currents give rather erratic responses (10) even when their form, amplitude and frequency are kept constant and the electrode contacts with the heart are meticulously controlled—details which do not seem to have been taken seriously by many experimenters. 3. Induction shocks of laboratory inductoria are notoriously erratic when examined on cathode-ray screens and maintenance of the same positions of the coils cannot be taken as a reliable index of constancy in faradic shocks.

A second method for determining the resistance of the ventricles to fibrillation consists in determining the duration of fibrillation in cats or the number of times that spontaneous recovery from fibrillation takes place. The first procedure was employed by Smith and Mulder (11). Using 2 seconds' tetanizing current, they noted that the average duration of fibrillation in 6 experiments was reduced from 26.3 to 11.8 seconds during stimulation of the accelerator nerves. Similar effects occurred after injection of epinephrine. However, Ettinger (12) found no relation between strength of current and duration of fibrillation in 40 normal cats; and the same effective current (minimal = 70 M.A.) applied at different times to the same animal induced fibrillation varying greatly in duration. We can confirm this with sine currents applied locally to a small ventricular area. For example, in one cat the duration in 8 consecutive tests ranged from 2 to 92 seconds. The second procedure was used by Van Dongen (7) who concluded that the resistance to fibrillation decreased after denervating the heart or use of various drugs.

A third method consists in comparing the threshold shocks which, applied during diastole, induce a premature contraction. The idea was suggested by one of us to McCord (13) who demonstrated in 1913 that the threshold is reduced by intravenous use of small doses of KCl, and it was again suggested to Mautz (14) who in 1936 demonstrated that topical application of KCl, metycaine or procaine reduces the irritability of the ventricles to single induction shocks. Unfortunately, the test is based on the premises that the induction of fibrillation is entirely a matter of myocardial irritability and that there is a relation between the diastolic stimulus necessary to evoke a premature contraction and the stimulus necessary to fibrillate. This remains to be demonstrated. It must also be clearly understood that if this proves to be a criterion for estimating the resistance to induction of fibrillation, it gives no information regarding the tendency of fibrillation to cease.

A fourth method employed by Beck and Mautz (15) was designed to gain information of such curative properties. It is based on the observation that revival of the dog's heart by use of brief A.C. countershock may not be successful, even after several trials. These investigators report that after procaine has been injected into the right ventricle, such a shock invariably proves successful after previous failure without its use. A large experience has convinced us that success in the use of the countershock method depends on the duration of fibrillation and certain details of technique. We have witnessed instances in which repeated trials failed to defibrillate the ventricles and, when we considered revival hopeless, still another shock was effective. Since this occurred in untreated dogs and since Beck and Mautz originally reported such apparent adjuvant action from application of procaine to the epicardial surface, we believe that their experiences may have been fortuitous.

A fifth procedure, recently suggested by Blumenthal and Tribe-Oppenheimer (16) is based on quantitative determinations of the amount of $BaCl_2$ which is just sufficient to fibrillate the perfused cat's heart. The procedure is obviously limited to a single test on one preparation. Using the method, these investigators noted that larger quantities of barium chloride are required after previous use of quinidine.

That a more critical quantitative test is required is indicated not merely by the logical criticisms which can be leveled against the several criteria suggested, but also by the contradictory conclusions which have been drawn from such tests. Thus, chloroform-adrenalin is a dangerous fibrillating combination according to Levy and Lewis (17) and many others (3), but it appears harmless according to the criteria of Meek et al. (2); epinephrine increases the resistance of the heart to fibrillating currents according to Smith and Mulder (11), but reduces it according to Hoff and Nahum (6). Action of the accelerator nerves favors induction of fibrillation according to Rothberger and Winterberg (18), van Dongen (7) and Braun and Samet (19), whereas it reduces the chance of fibrillation according to Smith and Mulder (11). Quinidine, which increases the resistance to fibrillation according to Levine (20), Blumenthal and Tribe-Oppenheimer (16) and many others, seems to make the ventricles more liable to spontaneous fibrillation after coronary occlusion, according to Moisset de Espanès (1), etc.

In 1938, one of us (21) suggested that if, as then seemed possible, a brief single shock causes fibrillation when applied during the vulnerable period of systole, it might be possible to measure the intensity of shocks of known form and duration and so establish a more quantitative *fibrillation threshold.* The likelihood that such a procedure would be applicable and adequate was greatly enhanced after it had been demonstrated (8, 9, 10) that brief shocks or the effective portions of longer stimuli cause fibrillation *only* when they are applied during the vulnerable period and have a mini-

mal effective strength. Since weaker shocks given during the vulnerable period elicit only a single premature beat, it seems probable that a fibrillation threshold so determined measures not merely the irritability of reactive fibers but also any other local state (partial refractoriness, block, altered conduction?) that may be necessary for the initiation of ventricular fibrillation. After it had been further shown that the dog's heart can be promptly and repeatedly revived within 15 to 30 seconds by a modification of the countershock method, successive tests were possible on the same animal. Thus, if it were possible to establish a reasonable constancy of thresholds after repeated revivals of dogs, it might be possible to determine the effects of chemicals, drugs, nerves, or other agencies on the fibrillation threshold.

It is apparent that use of such a quantitative criterion required a searching preliminary study *a*, of the most suitable and easily measurable electric shocks and means for applying them during an occasional vulnerable period, and *b*, of the experimental conditions necessary to keep the fibrillation threshold constant enough to enable recognition of changes induced by various agents. The present communication deals with these problems and analyzes the reactions after procaine, as an example of the magnitude of change which may be expected.

Choice of electrical stimuli and expression of fibrillation thresholds. While previous reports (8, 9, 10) have purposely been limited to qualitative aspects of stimuli and their temporal placement in a cardiac cycle in order to cause fibrillation, we have, during the course of the past year's experimentation, given considerable attention to quantitative possibilities of various stimuli.

It is obvious from data presented in previous papers that confusion can only be avoided by utilizing shocks which are short enough to fall entirely within the vulnerable period of late systole (ca 0.05 sec.). Experience has indicated that, for quantitative work, bipolar is preferable to unipolar stimulation, owing to changing contacts of the beating heart either with a posterior indifferent electrode or with the surrounding tissues, in the event the indifferent electrode is placed elsewhere in the body.

Of the many types of unitary electrical stimuli tested, we have found short rectilinear shocks, 0.01 to 0.03 second in duration, most convenient. Lower voltages can be employed and the danger of current spread is greatly diminished. The durations can be set at the start, but once decided upon, must not be changed during the course of an experiment. In this way, the comparative fibrillation thresholds may be simply expressed in milliamperes, measured from optical records inscribed by a calibrated oscillograph in circuit with the electrodes and stimulator, as previously described.

METHODS. The method for generating shocks of different strength and

for applying them successively in opposite directions through nonpolarizable Ag-AgCl electrodes was described in a previous paper (9). However, other procedures could obviously be used quite as well. As in previous work, the shock was applied every sixth beat of the ventricles, in order to allow time for restoration of cardiac irritability and pressure equalization after premature systoles. The shock was advanced or retarded with reference to a cardiac cycle by setting the tempo of the stimulator very slightly out of phase with the heart beat. This was tested by applying very weak shocks (ca 1 M.A.) to the ventricle until a whole series (10 to 15) caused no effect and another similar series all invoked premature contractions. A typical record reproduced as figure 1 shows that the shocks in the three upper records fall progressively later in systole and those of the lower record progressively later in diastole. If sufficiently strong, the shocks in the third record should each fibrillate. Such tests were then repeated with shocks increased in steps of about 4 or 5 M.A. until fibrillation was produced. Figure 2 illustrates graphically the effects obtained on several normal dogs.

Immediately after induction of fibrillation, padded electrodes were applied to the heart and a series of 4 to 7 weak A.C. shocks administered at about 5 second intervals, a process we call serial defibrillation. After a suitable interval (generally 15–20 min.) the test was repeated. It is surprising how accurately and precisely heart rate and blood pressure are generally reëstablished after repeated fibrillations.

Coefficients affecting the constancy of fibrillation thresholds. In view of the fact that the determination of every fibrillation threshold takes approximately 30 minutes and that experiments designed to study the effect of various agents or processes must therefore be prolonged for 5 or 6 hours, it became necessary to determine the constancy of such responses and to avoid all the influences which might possibly affect the threshold. Some of these we have demonstrated to be of importance; others had better be taken into account despite lack of such evidence. Among these coefficients, the following have so far been controlled as far as possible.

1. *Reasonable constancy in temperature of the ventricular surface.* Since the reactivity of tissues is definitely affected by temperature and since de Boer (22) criticized Lewis' observations on ventricular conduction on the ground that the exposed surfaces were allowed to cool, investigators have been worried by temperature changes of the cardiac surfaces, even to the extent of building special protective housings in which the operators' hands could be inserted (23). Incidentally, those who employed such protections did not demonstrate that they actually kept surface temperature the same. For this reason, we determined the surface temperature of the heart by aid of thermopiles in a considerable number of dogs under artificial respiration and with the heart exposed. Variations

up to a maximum of 1.5°C. were found in different regions; but over many hours, any one spot examined changed only a few degrees. Thus, in an experiment of January 9, 1940, a temperature of 32°C. was maintained from 1:50 to 4:29 p.m., after which it fell gradually to 31.1°C. by 5:30 p.m., and, in another experiment on January 10, 1940, the surface temperature of an identical spot decreased progressively from 32.3°C. at

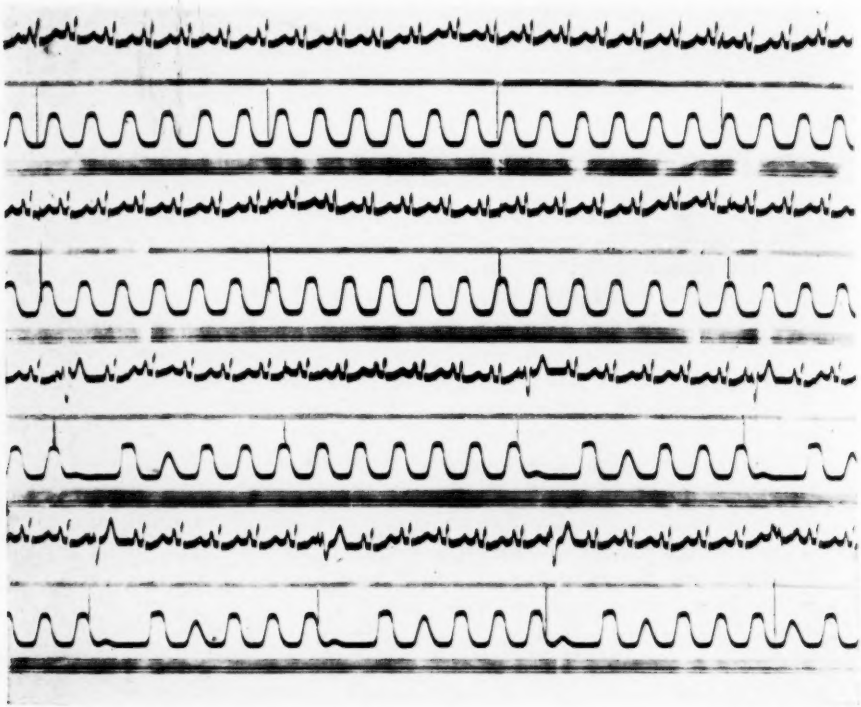

Fig. 1. Upper record E.C.G.; middle, D.C. shock; lower, left ventricular pressure. Note progressive advance in placement of very weak shock with regard to cardiac cycle.

1:05 p.m. to 30.3°C. by 6:10 p.m. We have not been able to discover any changes in threshold with variations in temperature of 3° or 4° as long as the temperature was above 30°C. If such exist, our criterion is not able to detect them.

We have also compared surface and intracardiac temperatures read on a thermometer inserted into the right auricle via a jugular vein and found a

fairly constant difference ranging from 1° to 2° C. in different hearts. When the animal cooled, these temperatures fell together and when heat was applied, they rose proportionately. We were, therefore, led to the conclusion that, owing to the abundant coronary blood supply, the surface temperature of the ventricles is controlled essentially by blood temperature which can be kept fairly constant by control of the heat beneath an animal; it is affected little by radiation, conduction, convection, and evaporation, provided these are not allowed to alter to an extreme degree. Consequently, it is not necessary to utilize elaborate protections to keep the circumambient air constant in experiments of this type. Recording of intracardiac temperatures is a sufficient indication of the trends of surface temperature.

2. *Stimulation of the same point.* The literature contains implications that some regions of the heart may be more responsive to fibrillation than others (24); but such inferences were drawn from use of prolonged tetanizing or direct currents. We have tested the reactivity of various points to D.C. shocks, 0.02 sec. duration, and occasionally found small but not significant differences. Nevertheless, it appears important to stimulate a definite spot in relation to distinguishable anatomical landmarks. The possibility looms large that the route of spread of the initial premature beat over muscle bundles is a factor which is involved in induction of fibrillation. This would naturally differ if different areas were excited. In our experiments, we always selected a region on the left ventricular surface in the angle formed by continuation of the ramus descendens and one of its lower branches spreading to the left. Thus, the chance always existed that the initial spread of the excitation wave would be to the right and left ventricles over the superficial sino-spiral fibers. Repeated stimulation of this area through rigid Ag.-AgCl electrodes so applied that they depress the surface a trifle does not seem to damage the heart even when applied for hours.

3. *Polarization, electrophoresis or anodal-cathodal changes in irritability.* These must naturally be prevented in such prolonged bombardment of a spot by short D.C. shocks. This was accomplished satisfactorily by careful plating of electrodes and meticulous reversal of current in successive shocks through our automatic pole-changer, as attested by conservation of the same recorded value of the stimuli in prolonged tests.

4. *Allowance of an adequate equilibration period after each fibrillation and revival.* Despite the prompt recovery from fibrillation (15–30 sec.), general avoidance of auricular fibrillation and restoration of beats dominated by a sinus pacemaker, the possibility still existed that such influences as temporary asphyxia, brief abolition of intracardiac pressure elevations, residual effects of the countershock, metabolic effects of the

fibrillating process itself, physicochemical changes in the blood during temporary failure of circulation, etc., may so alter the excitability and conductivity of the myocardium that no constancy in fibrillation threshold would be found in repeated trials. In our earlier tests, threshold determinations were made at irregular intervals of 3 to 10 minutes after restoration of normal beats; and we did find significant variations. We then extended the recovery period to 15 to 20 minutes with the hope that such a more extended and constant equilibration period would give better results.

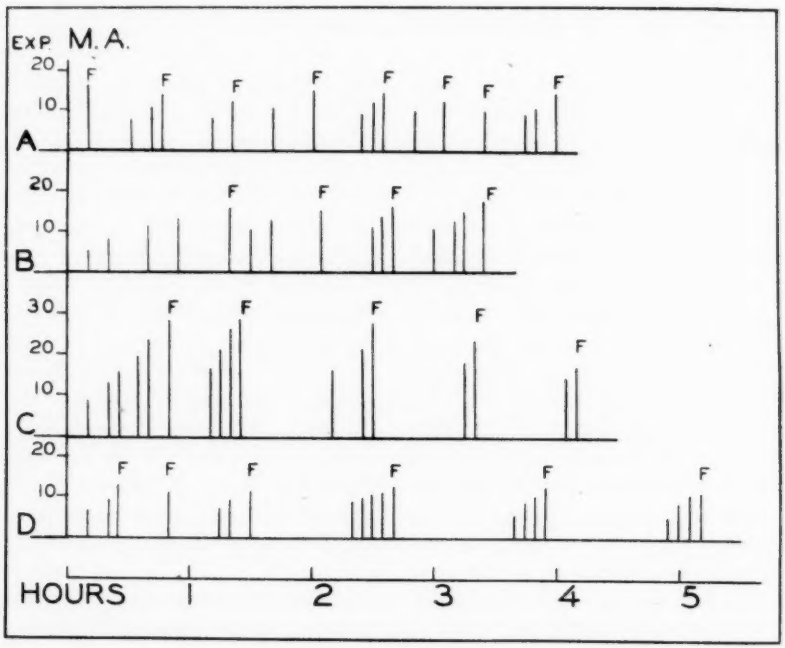

Fig. 2

Doing this we found in the majority of experiments that consistent thresholds were obtainable. Representative experiments extending over 4 or 5 hours are plotted in figure 2. The vertical lines indicate M.A. values of 0.02 to 0.03 second rectilinear shocks used to test the fibrillation threshold; the values represented by lines labeled F causing fibrillation, the others not. Experiment A shows a slight tendency for the threshold to decrease and in experiment C it decreases somewhat more. In experiments B and D, a remarkable degree of constancy obtains. Many such experiments lead to the conclusion that gradual, slight and progressive

variations in fibrillation threshold after use of a drug or operation of a physiological action cannot be taken as a change in resistance to an electric shock; but if the thresholds change abruptly, this seems to be significant.

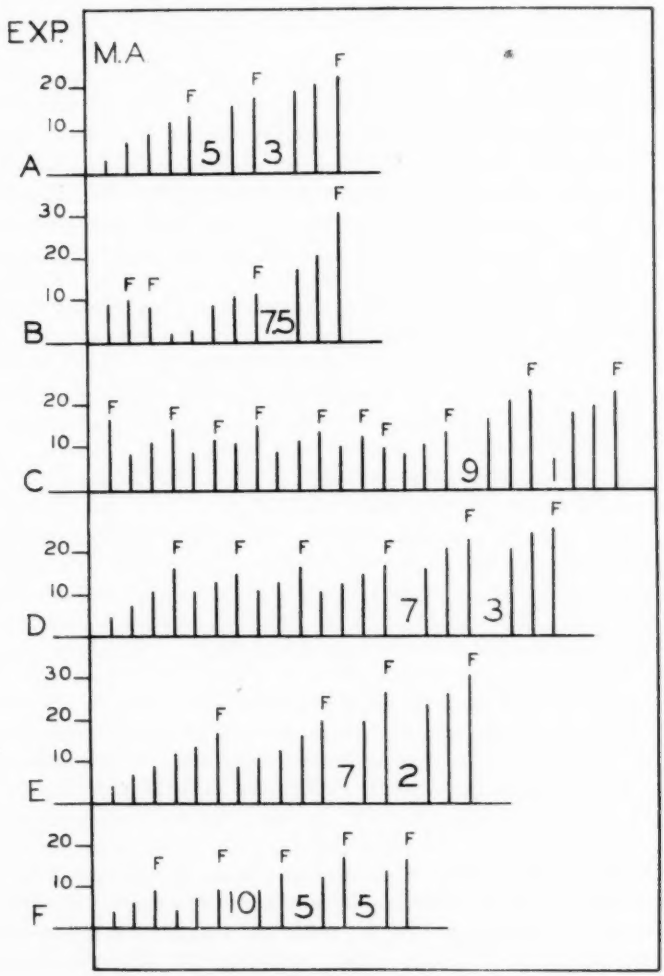

Fig. 3

While we make no claims of having established a threshold of fibrillation which compares with the refinement of stimulation thresholds used in studies of nerve-muscle irritability, we do believe that we have traveled

significantly toward such a goal. When we consider the difficulties of not dealing with a static state of irritability, of requiring introduction of a stimulus at a definite moment of the cardiac cycle, of coping with the significant changes in environment which even a short period of fibrillation may produce, etc., it is not surprising that a greater degree of refinement has not been achieved.

The effect of procaine. That procaine may affect the myocardium was indicated by the experiments of Shookhoff (25) and that epicardial application increases the threshold of induction shocks necessary during diastole for production of premature contractions was demonstrated by Mautz (14). Since the strength of tetanizing currents necessary to produce fibrillation also needed to be increased, he concluded that procaine increases the resistance to fibrillation. Confirmatory evidence as regards the protective action of a simultaneous intravenous dose of 8 to 10 mgm./kilo against benzol-adrenalin fibrillation and of 5 mgm. against chloroform-adrenalin fibrillation was presented respectively by Shen (4) and Shen and Simon (3). Burstein and Marangoni (15) found that 5 mgm. per kilo given previously exerts a protecting action against cyclopropane-epinephrine fibrillation. We have already indicated why we regard these results suggestive but not conclusive.

In 6 dogs, after previous determination of several fibrillation thresholds, 8 to 10 mgm./kilo procaine hydrochloride were injected, usually in divided doses. The results are shown graphically in figure 3 in which the numerals indicate the doses used in milligrams per kilo. In every instance, a significant increase in threshold occurred, the most convincing being experiments B, C, D and E. In experiment E, for instance, a shock 0.02 second in duration fibrillated with a strength of 17 and 20 M.A.; but after slow infusion of 7 mgm. procaine per kilo, 20 M.A. did not fibrillate, but 27 M.A. did. After an additional administration of 2 mgm. per kilo, 24 and 27 M.A. were ineffective but 31 M.A. caused fibrillation. We found no instance in which the heart became entirely refractory to fibrillation and repeated tests showed that such a shock must still be given during the vulnerable period in order to fibrillate; at other times it does not fibrillate. In short, such observations indicate that procaine raises the fibrillation threshold during the vulnerable period as it does the threshold for premature systoles during diastole. It is not a preventive. Revival by countershock occurred promptly, but since this always occurred in normal hearts, no deductions could be drawn regarding the adjuvant action of procaine.

SUMMARY AND CONCLUSIONS

The methods used for determining variations in the sensitivity or resistance of the ventricles to fibrillating agents are briefly reviewed and reasons presented why they are inadequate.

A new quantitative measure for the "fibrillation threshold" of the ventricles is proposed and tested. We believe it takes into account the irritability of non-refractory myocardial fractions during the vulnerable phase of systole, and also any local state that may be necessary for the initiation of ventricular fibrillation. The procedure consists in measuring the current strength of brief D.C. shocks of constant duration (0.01 to 0.03 sec.) which are just able to induce fibrillation when applied during the vulnerable period of late systole to any fixed region of the ventricular surface.

The coefficients which modify the constancy of such thresholds were studied and it was established that, despite repeated fibrillations and defibrillations, the threshold need not vary significantly and does not change abruptly over a period of 4 to 5 hours in untreated dogs. To obtain such relative constancy of fibrillation thresholds, the following conditions were observed: 1. Revival of the heart from fibrillation in less than 30 seconds. 2. Maintenance of the animal's blood temperature within 1 to 2°C. during the course of an experiment, which essentially determines surface temperature as well. 3. Stimulation at the same spot by D.C. shocks applied in alternate directions through non-polarizable electrodes. 4. Allowance of an equilibration period of not less than 15 minutes between one fibrillation and resumption of tests.

The applicability of the quantitative method was tested with reference to procaine which is reputed to increase the resistance of the ventricle to certain fibrillating agents. In six dogs, an increase in threshold, definitely beyond normal bounds was found; after treatment with procaine, a brief shock still fibrillates only when it is applied during the vulnerable period. We conclude that procaine raises the resistance of the ventricles to fibrillation but does not prevent its occurrence.

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THE EFFECTS OF MYOCARDIAL ISCHEMIA ON THE FIBRILLATION THRESHOLD—THE MECHANISM OF SPONTANEOUS VENTRICULAR FIBRILLATION FOLLOWING CORONARY OCCLUSION[1]

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In a previous communication (1) we suggested that the current strength of brief D.C. shocks (ca 0.01 to 0.03 sec.) which are just able to fibrillate the dog's ventricles when applied during the vulnerable period, to a constant small spot of the ventricular surface offers a quantitative measure of the "fibrillation threshold."

The validity of this criterion would be supported and a more general applicability would be suggested if it were found that such a threshold decreases significantly in conditions in which a greater tendency to fibrillation exists. It will probably be generally accepted that this occurs after a large part of a ventricle has been rendered totally ischemic by coronary occlusion.

The present communication presents evidence that the fibrillation threshold is materially reduced after coronary occlusion and suggests a hypothesis which accounts for the spontaneous fibrillation that so frequently supervenes.

PROCEDURE. Dogs weighing 8 to 10 kilos and anesthetized by morphine and sodium barbital were used. The chest was opened under artificial respiration and the heart was suspended in a pericardial cradle. The ramus descendens was dissected for a length of about 1 cm. and prepared for clamping. Left intraventricular pressure, an E.C.G. (lead III), and a quantitative record of the shock through a calibrated G.E. oscillograph were simultaneously recorded. In this way, the strength, duration and moment of application for each stimulus were registered. The stimulus was always applied to a spot which would be in the ischemic territory during coronary occlusion. The duration of the rectilinear D.C shocks (0.01 to 0.02 sec.) and the locus of application were not changed during

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[2] Fellow of the Belgian-American Educational Foundation.

the experiment. The method for creating the stimulus and for advancing it in relation to the heart cycle, as well as the general precautions required to insure constancy in threshold, were described in previous communications (1, 2).

After several tests on the normal heart had established the fibrillation threshold with certainty, the *ramus descendens* was occluded. One or two minutes later, a test for another threshold was started. Such a short interval after occlusion was selected because it has been shown by Tennant and Wiggers (3) that, by this time, the area involved has been affected by the anoxia to the extent that it fails to shorten. Furthermore the ventricles are more easily defibrillated by countershock and the chance for development of spontaneous premature systoles or fibrillation is much less than it is later. Nevertheless, in order to differentiate a possible spontaneous fibrillation from that due to the artificial shock, it was necessary to take records throughout the period of stimulation. Only those fibrillations which were definitely related to a shock (figs. 2-3) were counted as fibrillation thresholds during coronary occlusion.

Immediately after fibrillation, the coronary clamp was released and the ventricles were rhythmically compressed for about 30 seconds in order to revitalize the ischemic tissue by an artificial coronary flow. In these experiments, the aorta was compressed by the second and third fingers, while the left ventricle was pressed rhythmically, thus sending most of the blood expelled into the coronary vessels. Then, a short series of weak A.C. shocks was sent through the ventricles. In general, a greater number was required and the time of revival was extended to 1 or 2 minutes.

After waiting for 20 minutes, a test was repeated while the normal coronary flow persisted. In this way, fibrillation thresholds were determined alternately with and without coronary occlusion.

Results. The general nature of the results in 7 different dogs is graphically charted in figure 1. The abscissae indicate the time scale, and the ordinates denote the strength of the stimuli. The durations of stimuli are given in the legend. Each separate vertical line indicates a strength of stimulus tested. Those resulting in fibrillation when vessels were not occluded are marked N.F.; those inducing fibrillation during occlusion of vessels are designated O.F.

We may profitably analyze experiment 3 in detail. The duration of the D.C. shock was 0.02 second throughout the experiment. In the first determination, a shock of 15 M.A. was required to fibrillate. After defibrillation, recovery, and a 15 minute pause, a threshold shock of 11.5 M.A. caused fibrillation. After still another defibrillation and wait, a third test gave a fibrillation threshold of 12.7 M.A. for the normal heart. After the usual recovery period, the *ramus descendens* was occluded, and 1 minute 30 seconds later, a shock was applied during every sixth normal

beat. The second shock of 5.2 M.A. so applied fortuitously fell during the vulnerable period and fibrillated the ventricle. The possibility of course exists that a still weaker shock might have done so, but it definitely indicates a decrease in threshold. After defibrillation and following another

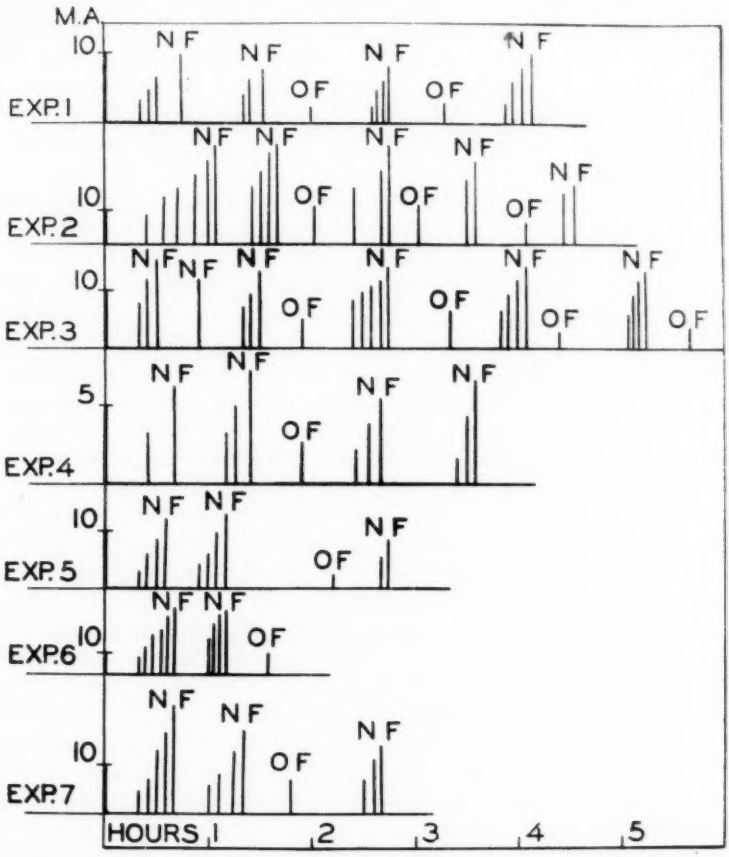

Fig. 1. Diagram indicating the strength of currents causing fibrillation in normal dogs (N.F.) and in the same animals after coronary occlusion (O.F.). Duration of stimuli as follows: Experiment 1—0.01 second; experiment 4—0.016 second; experiments 2, 3, 5, 6, 7—0.02 second.

15 minutes' equilibration period, the ventricle was again stimulated without occluding the coronary artery. As shown in the graph of figure 1, the stimulus value had to be increased progressively to 13.9 M.A. before fibrillation resulted from a shock given during the vulnerable period.

The next test, made during coronary occlusion, showed that a stimulus of 6.4 M.A. induced fibrillation 1 minute 48 seconds after occlusion. Once more, after revival and a recovery period, the fibrillation threshold came back to the same level, i.e., 13.9 M.A. A subsequent test during a new period of occlusion showed that a stimulus of 2.9 M.A. was sufficient to fibrillate the ventricle. After recovery, the normal heart again required 11.5 M.A. to fibrillate, and finally, during a last occlusion, a 3 M.A. shock sufficed to fibrillate 1 minute 52 seconds after occlusion. The repeated alternation of threshold values during and without coronary occlusion leaves no doubt but that ischemia significantly reduces the fibrillation threshold.

Similar reactions were found in the other six dogs, although the experiments were not continued as long. Of supplementary interest are the recoveries of threshold following ischemia, fibrillation and revival. In experiments 1, 3 and 4, the threshold practically returned to that established at the beginning; in experiments 2 and 5 it was decreased somewhat and in experiment 6, no determination could be made after coronary occlusion, since recovery from fibrillation was unsuccessful. Such failure to revive the heart by preliminary massage and serial countershock, particularly after prolonged coronary occlusion, was experienced a number of times. This stresses the greater difficulty of restoring a normal functional state to myocardial fractions subjected to prolonged ischemia.

Critique of interpretations. Tennant and Wiggers (3) demonstrated that the ischemic area no longer shortens one minute after coronary occlusion but expands passively with the rise of intraventricular pressure created by contraction of other myocardial fractions. Since the ischemic myocardium, therefore, does not contribute to the pressure elevation, the question arises whether the ventricular pressure curve still serves as a criterion of the vulnerable period of the region stimulated. The thought is reënforced by observations initially reported by Orias (4) that the contour of the pressure curve is altered and the duration of systole measured therefrom is decidedly shortened. Furthermore, the possibility exists that the refractory periods of fractions in the ischemic area may either be prolonged or abridged.

Records such as are reproduced in figures 2 and 3 not only confirm such suspicions but as a matter of fact give rise to them. It will be noted that the pressure curves are decidedly more peaked than under normal conditions and that the periods of contraction are short. Moreover, a stimulus falling at various moments in relation to the peak induces fibrillation. Thus, while a hasty analysis of figure 2 might properly assign the incidence of the stimulus to the vulnerable phase as previously defined, it also falls rather early in systole; in fact, it coincides with the rise of the T wave of the E.C.G. In figure 3, the fibrillating shock falls definitely on the descending

limb of the pressure curve. The oscillographic tracing shows that fibrillation was induced by a weak shock in all instances.

We must conclude that the span of the vulnerable period in the ischemic myocardium does not bear the normal relationship to the end of systole, demarcated by the pressure curves.[3] Unfortunately, no other record serves as a better guide. The E.C.G. waves, which suggest themselves,

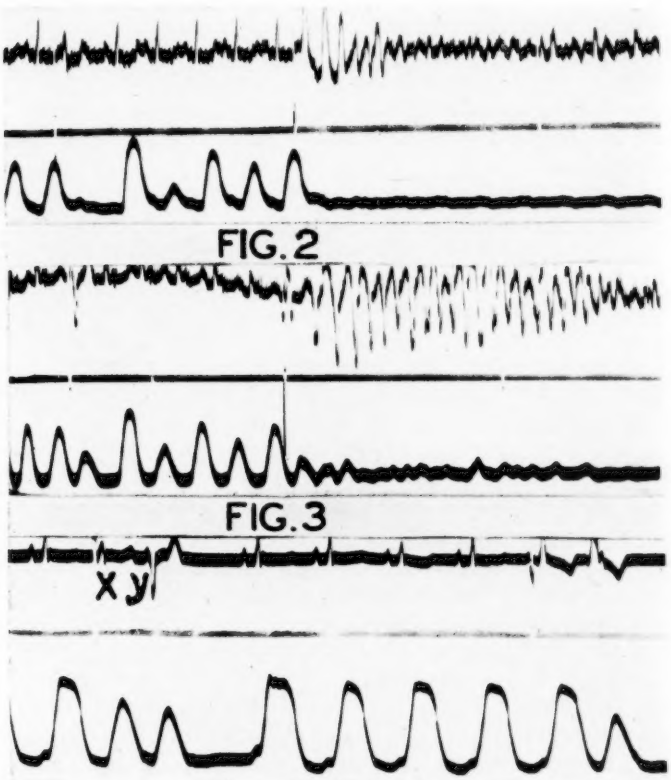

Fig. 4

frequently change as regards the RST segment or T wave and with the abreviation of systole offer no better landmarks. However, it is clear that, even if the vulnerable moments of the ischemic area bear a very fortuitous relation to the pressure curve created by other fractions, it still serves the useful purpose of differentiating fibrillation due to the shock from that

[3] The probability of a similar lack of relation to pressure curves due to ectopic ventricular stimuli has been previously discussed (2).

which may arise spontaneously. Repeated observations indicated clearly that a similar fibrillation is never induced after the pressure wave has returned to its base line. The only difference noted between the reaction of the normal and ischemic myocardium to brief diastolic shocks is the occasional occurrence of two instead of one premature beat; fibrillation never occurs. As illustrated in figure 4, the second of these ectopic beats, Y, may have a different configuration from the first, X, and apparently arises spontaneously from another focus. This, of course, is consonant with the general impression that ischemia enhances the tendency to develop ectopic foci. Apparently it is not even necessary to observe the strict incidence of the shock during the pressure curve, as we have done, in order to compare thresholds of brief shocks which fibrillate before and after coronary occlusion.

Physiological state of the ischemic myocardium. Experimental observations of this part of our investigations have again raised the question as to the physiological state of the potentially infarcted area. In their study of coronary occlusion, Tennant and Wiggers (3) showed that despite a lack of evidence of shortening, the ischemic area remains irritable to induction shocks, and that impulses spread as in normal hearts to both ventricles. Our observations showed that, similarly to normal hearts, an effective brief stimulus induces fibrillation only when applied during the vulnerable period (redefined in relation to the pressure curve), and that the threshold value is significantly reduced. We cannot say whether this signifies solely an enhanced excitability of fractions during the early moments of the non-refractory state or involves in addition a greater asynchronism in passage of myocardial elements out of the refractory state, decrease in refractory period, or differences in conduction which facilitate reëntry.

The observations of Tennant and Wiggers (3) that the ischemic region no longer shortens but stretches, can perhaps not be interpreted as complete failure of the effort of contraction, but rather that the strength of such effort is not sufficient to overcome the stretching force of rising ventricular pressure. In other words, contractions become feeble; they do not stop. The suggestion of Tennant and Wiggers that dissociation of irritability and contractility is demonstrated, must be retracted. In support of these statements we may cite our observations that when the ventricles fibrillate spontaneously or after a brief shock applied to the ischemic area, the whole region participates in fibrillary contractions. Presumably, with removal of the high intraventricular pressures, the contractile processes are able to manifest themselves. Likewise, with application of a series of countershocks, this area comes to rest with the remainder of the myocardium. The reason that revival of vigorous coördinated beats is more difficult following a period of coronary occlusion, is partly that this region remains ineffective in elevating ventricular pressures and partly that so many ectopic beats arise that the heart easily reverts to fibrillation.

The mechanism of "spontaneous" fibrillation after coronary occlusion. We are not aware of the existence of any clear statement with reference to the mechanisms which abruptly initiate fibrillation during coronary occlusion. Descriptions stress the variable periods after which it may occur and vaguely enumerate coefficients which seem to affect the time interval before fibrillation develops. The fact that it is preceded by numerous isolated groups or trains of premature beats is well known. But the question as to their intimate relation to fibrillation and the reasons why they sometimes eventuate in fibrillation and at other times not, is studiously—and perhaps wisely—avoided.

Certain observations and logical conceptions to which they have given rise tempt us to become more venturesome. We have submitted evidence in this and preceding reports that, in order to induce ventricular fibrillation, we must have an effective stimulus, which may be of brief duration and initially operate in a localized region, but which must fall during a vulnerable period. This we interpret to represent an irregular and asynchronous passage of somewhat adjacent fractions from a refractory to a non-refractory state. During spontaneous fibrillation the stimuli obviously arise within the myocardium. Evidence that more than one focus may release them after coronary occlusion is supplied by the frequent occurrence of premature beats, as a prelude. According to our conception, fibrillation would result if one such ectopic impulse fell during the vulnerable period of a normal beat or that of a premature beat induced from another focus. In addition to such proper spacing of impulses, which must be a frequent normal occurrence, it is important that the strength of the stimulus be adequate. This can either be achieved by increasing the intensity of the shock, as in the production of fibrillation in normal hearts by brief, strong stimuli, or by enhancing the myocardial irritability so that a spontaneous impulse acquires an effective fibrillating value. We suggest that this happens in spontaneous fibrillation following coronary occlusion.

According to this hypothesis, acute ischemia introduces dual coefficients. 1. It gives rise to many ectopic foci which release stimuli which are subthreshold as far as induction of fibrillation in the normal myocardium is concerned, and 2, it increases irritability sufficiently so that these stimuli become effective when they fall during the vulnerable phase.

SUMMARY

Alternate determinations of the fibrillation threshold of ventricles with a normal blood supply and during brief coronary occlusion were repeatedly made on the same animal. Rectilinear shocks 0.01 to 0.02 second in duration were applied to an identical area of the left ventricle. The milliampere value of a shock which, when applied during the vulnerable phase, induced fibrillation, served as the quantitative measure of the fibrillation threshold.

Significant reduction in the current strength required to fibrillate was noted during coronary occlusion, e.g., a decrease from 13.9 to 3 M.A. in one experiment. The vulnerable period of the ischemic region does not bear a normal relation to the ventricular pressure curve because the contraction of fibers in the ischemic area does not contribute to the pressure elevation and because the form and duration of the pressure curve are altered. Consequently, stimuli coinciding with the descending limb fibrillate, but those which fall on the horizontal portion of the pressure curve are ineffective in this respect.

The observations of Tennant and Wiggers that the ischemic area no longer shortens but stretches during systole cannot be interpreted as a complete failure of the effort of contraction, but rather that the strength of effort is insufficient to overcome the stretching force of rising intraventricular pressure. When this force is removed during fibrillation, contractile waves are able to manifest themselves again.

Since ischemia reduces the fibrillation theshold to artificial stimuli and also causes formation of ectopic centers, the theory is put forward that spontaneous fibrillation during coronary occlusion is precipitated because such ectopic stimuli now become of threshold value for the hyperirritable myocardium and any one is capable of inducing fibrillation when it falls during either the vulnerable period of a normal beat or that of a premature beat excited from another ectopic center.

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THE EFFECT OF THYROIDECTOMY ON SERUM CHOLESTEROL AND BASAL METABOLIC RATE IN THE RABBIT

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The relationship between the B.M.R. and the level of serum cholesterol in hypothyroidism has been studied by a number of workers. It has been demonstrated in human beings that the B.M.R. rises as the serum cholesterol falls, and vice versa (1). This is best shown by the changes which occur when treatment with thyroid hormone is first begun or when it is discontinued. In children in whom measurement and evaluation of B.M.R. is often difficult, studies of serum cholesterol therefore are of importance as an additional method for diagnosis (2).

It seemed of interest to supplement by animal experimentation our knowledge gained through studies on normal and hypothyroid children. The rabbit was selected as an experimental animal because it has no accessory thyroid tissue and total thyroidectomy can be performed without risk of hypoparathyroidism. It has been shown by Marine and Lenhart (3) that on the fifth to seventh day after thyroidectomy the B.M.R. of the rabbit begins to fall from normal values of 477 to 607 cc. O_2/kgm/hr. and reaches its lowest level of 348 to 355 cc. O_2/kgm/hr. between the 20th and the 30th day after thyroidectomy. Schenk (4) reports similar results.

A rise in blood cholesterol after thyroidectomy has been reported by several investigators (5, 6, 7, 8, 9, 10). The changes reported differ widely, however. Westra and Kunde (8), for instance, report an increase from a concentration of 74 to 94 mgm. per cent in the normal to a postoperative level of 222 to 227 mgm. per cent. This is an average increase of 267 mgm. per cent. Turner, Present and Bidwell (10) find an average increase of only 19 per cent after thyroidectomy. Yoshimura (11) showed that the hypercholesterolemia after the removal of

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the thyroid is only transient. The concentration of the blood cholesterol reached the maximum one to two weeks after operation, and returned to normal in five to seven weeks.

It seemed of interest to follow both the B.M.R. and the serum cholesterol in the same animals. Cholesterol was determined in the serum rather than in the whole blood as changes are more marked in the serum or plasma than in the whole blood.

MATERIALS AND METHODS. Rabbits of an inbred strain of Dutch and American Blues were used. All except one (no. 31) were males. They were kept in separate metabolism cages and fed on a stock diet described by Hyde (12). (Formula of diet: coarsely ground wheat 5000 grams, chopped alfalfa 5000 grams, flaxseed meal 250 grams, calcium carbonate 85 grams, sodium chloride 55 grams.)

Thyroidectomy was performed under ether anesthesia. At the conclusion of the experiments the absence of thyroid tissue was verified by autopsy.

The B.M.R. was measured with the simple apparatus for metabolic measurements described by Tainter and Rytand (13). The apparatus was built on a larger scale than the original so that a rabbit could be conveniently placed in the chamber and changes in volume from 10 to 250 cc. could be recorded. The chamber was large enough to permit air to be used instead of oxygen. The data obtained with this apparatus compared well with those of Marine and Lenhart (3), who used a Haldane apparatus.

Blood was taken from the ear vein and the total cholesterol was determined in the serum by the method of Bloor adapted to the Evelyn colorimeter (14).

Serum cholesterol and B.M.R. after thyroidectomy. Observations were made simultaneously on the serum cholesterol and B.M.R. of 15 rabbits over periods of 16 to 19 weeks following total thyroidectomy. Prior to operation the values for the normal were determined and in many instances repeated observations were made.

During the first six weeks after operation there was a gradual decrease in B.M.R. to about 40 per cent below the pre-operative level. After thyroidectomy the serum cholesterol showed a sharp initial rise varying from 81 to 340 per cent (average 171 per cent) above the pre-operative level. After the first rise the serum cholesterol fluctuated markedly, finally becoming stabilized after about 12 weeks at a value 14 per cent to 221 per cent (average 80 per cent) above the pre-operative level. On figure 1 all single determinations are recorded as dots. This figure illustrates the great variations of serum cholesterol after thyroidectomy in contrast to the gradual decrease in B.M.R. On figure 2 three representative experiments of this series are shown.

Repeated determinations showed that the serum cholesterol never fluctuated more than 36 mgm. per cent in any rabbit before operation. This corresponds closely to the observation of Horiuchi (15), who reported values of plasma cholesterol of 40 to 100 mgm. per cent in normal rabbits, with individual fluctuations less than 30 per cent. The instability of the serum cholesterol following thyroidectomy was in marked contrast to this. During the first 12 weeks after operation we found fluctuations as great as 224 mgm. per cent in some rabbits. After the 12th week there was apparently less tendency for the cholesterol to

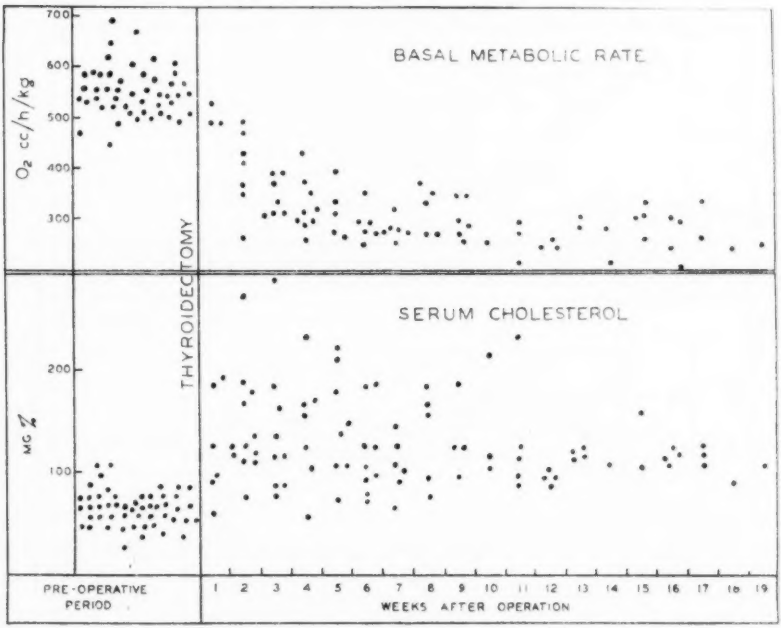

Fig. 1

fluctuate since the greatest variation observed was 55 mgm. per cent. However, the number of observations in later weeks was too small to warrant definite conclusions.

The instability of the serum cholesterol after thyroidectomy suggests that the thyroid is an important factor in regulating blood cholesterol. The marked fluctuations which occur immediately after operation could be explained by a removal of the regulatory mechanism. It is conceivable that after several weeks a new regulatory mechanism is established in which the thyroid is no factor. The fact that there is a gradual and

regular decrease of the B.M.R., whereas there are wide fluctuations of serum cholesterol after thyroidectomy, indicates that there is no direct relationship between B.M.R. and blood cholesterol, although both are influenced by the thyroid. That changes in the blood cholesterol level are not directly related to the metabolic rate has been shown previously in human beings by the fact that dinitrophenol raises the B.M.R. without causing a drop in serum cholesterol (16, 17).

The sensitivity of normal and thyroidectomized rabbits to the administration of thyroxin. It has been shown by P. E. Smith and his associates (18) that the effect of thyroid extract on the metabolism of thyroidec-

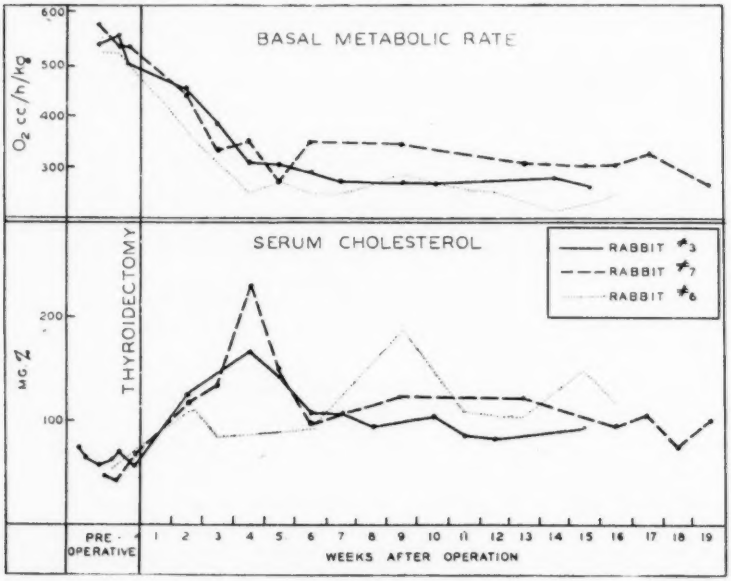

Fig. 2

tomized rats is much greater than on that of normals. Similar observations have been reported in dogs (19, 20). In human beings a single dose of thyroxin causes a greater effect in untreated hypothyroid patients than in treated patients or in normal individuals; and, in addition, the effect lasts over a considerably longer time (21, 22).

Our experiments on rabbits showed the same contrast between the normal and the hypothyroid animal. Thyroxin (Roche-Organon) was injected subcutaneously after a base line had been established. These experiments are summarized in table 1. The basal metabolic rate was used as an index of thyroid effect rather than the serum cholesterol

level because of the great fluctuations in serum cholesterol which occur in hypothyroid rabbits. The data in table 1 indicate that the effect of thyroxin is much greater in hypothyroid than in normal rabbits. It is further to be seen from the last column of this table that the lethal

TABLE 1

Sensitivity of normal and thyroidectomized rabbits to the administration of thyroxin

RABBIT NUMBER	DOSE OF THYROXIN	MAXIMUM RISE OF B.M.R.	DAYS AFTER WHICH MAXIMUM IS REACHED	REVERTED TO NORMAL AFTER	MAXIMUM DECREASE IN CHOLESTEROL	
			Normal rabbits			
	mgm.	*per cent*		*days*	*mgm. per cent*	
3	0.5	9	2	3	10	
16	0.8	13	2	3	0	
6	2.0	11	4	9	0	
1	3.0	17	3	6	32	
3	5.0	27	2	7	15	
7	5.0	35	3	8	2	
5	5.0	25	2	4	8	
8	5.0	28	3	5		
2	5.0	44	2	8	12	
9	5.0	31	4	6		
8	7.0	6	3	4		
43	10.0	69	3	6	32	
42	10.0	38			14	Died on 2nd day
43	20.0	79	3	5	28	
			Thyroidectomized rabbits			
14	0.5	32	2	4	53	
13	0.5	89	4		21	Died on 7th day
14	0.8	44	3	8	38	
6	1.0	92	8		30	Died on 13th day
10	1.0	59			56	Died on 2nd day
22	2.0	122	5	9		
21	5.0	174	4	10	56	
3	5.0	65			57	Died on 2nd day
21	5.0	93	6		26	Died on 8th day
26	10.0	78			91	Died on 4th day
27	10.0	47			30	Died on 3rd day
31	10.0	96	4	9	36	Died on 15th day

dose of thyroxin for the hypothyroid rabbit may be smaller than that for the normal. All normal animals survived except one (no. 42) injected with a single dose of 10 mgm. In the hypothyroid group doses as low as 0.5 mgm. sometimes proved fatal. One thyroidectomized rabbit (no. 31) died 6 days after the B.M.R. and serum cholesterol had

reverted to normal. Death was probably due to thyroxin poisoning, as no other cause could be discovered at autopsy.

A marked increase in creatinuria is caused in hypothyroid children by a single dose of thyroxin (22). Creatine studies were unsatisfactory in most rabbits as the creatine excretion showed marked fluctuations. In a few rabbits having fairly constant creatine excretion it was found that small doses of thyroxin (0.5 mgm.) caused increased creatinuria in thyroidectomized animals, whereas large doses (5.0 mgm.) were required to cause any effect in normal animals. For instance, in a thyroidectomized rabbit (no. 14) a sharp rise in creatine excretion (48 hr. period) from a level of 8 mgm. to a level of 48 mgm. following injection of 0.8 mgm. of thyroxin was observed, whereas the creatine excretion of the control animal (no. 16) was not affected, staying on its level of 15 mgm. creatine excreted (48 hr. period).

Discussion. It is generally recognized that there are marked differences in the cholesterol metabolism of human beings and rabbits. In spite of this fact, the studies reported here show similarities to certain observations made by the writers (2, 22, 23) upon hypothyroid children.

1. In a group of 21 untreated hypothyroid children the serum cholesterol varied from 145 to 660 mgm. per cent compared to values ranging from 98 to 308 mgm. per cent in normal children. In the hypothyroid rabbits the range was from 70 to 290 mgm. per cent compared to from 33 to 107 mgm. per cent in normal controls. In both human beings and rabbits the ranges of the hypothyroid and normal groups overlap.

2. In following the cholesterol level in given individuals over long periods, greater spontaneous fluctuations were encountered in hypothyroid than in normal children. The fluctuations in hypothyroid patients were as great as 200 mgm. per cent, whereas in normal children fluctuations never exceeded 83 mgm. per cent and generally were much smaller. In thyroidectomized rabbits individual fluctuations were as great as 224 mgm. per cent, whilst in the normal the greatest fluctuation found was 36 mgm. per cent. The marked instability of the serum cholesterol in hypothyroid individuals may account for the low values which are frequently observed in hypothyroidism.

3. The sharp rise in serum cholesterol after thyroidectomy in the rabbit is similar to the rise observed in hypothyroid children after withdrawal of thyroid medication. In normal children withdrawal of thyroid medication has no such effect. In some of the hypothyroid children serum cholesterol rose to a peak after withdrawal of medication, and then dropped to a lower level (23). This rise and fall is very similar to that observed in rabbits after thyroidectomy. It is quite possible that such peaks in the concentration of cholesterol in the serum might have been observed more frequently in hypothyroid children if thyroid

treatment had been withheld for a longer period of study. This, however, did not seem justifiable from a therapeutic viewpoint.

4. The greater sensitivity of hypothyroid rabbits in comparison to normals to a single injection of thyroxin, as evidenced by a greater rise in B.M.R., drop in serum cholesterol, and in some cases increased creatine excretion, is very similar to the greater sensitivity observed in hypothyroid children (22).

Although both the studies on rabbits and those on children produce strong evidence of the relationship between the thyroid gland and cholesterol metabolism, they shed no light on the mechanism of the action of the thyroid hormone on the serum cholesterol level.

SUMMARY

Thyroidectomy in the rabbit is followed by a sharp rise in serum cholesterol varying from 81 to 340 per cent (average 171 per cent) above the preoperative level. After the first rise the serum cholesterol fluctuates markedly, finally becoming stabilized after about 12 weeks at a value 14 to 221 per cent (average 80 per cent) above the base level. The basal metabolic rate does not show marked fluctuations, but decreases gradually in six weeks to about 40 per cent below the preoperative level. Thyroidectomized rabbits are more sensitive than normals to a single injection of thyroxin, as can be shown by following the changes in the basal metabolic rate, serum cholesterol and creatine excretion. The relations of these findings in rabbits to similar studies in human beings are discussed.

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WATER DISTRIBUTION AND SEXUAL SKIN OF THE BABOON

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The sexual skin of the perineal region of many primates undergoes rhythmic alterations in size as well as in color throughout successive menstrual cycles. This phenomenon has been studied by several workers, especially by Zuckerman (1937) who has summarized the evidence in support of the theory that the post-menstrual turgescence is due to the increased secretion of estrogens, and that their sudden post-ovulatory suppression is responsible for the rapid deturgescence. Gillman (1935) reported a long series of observations on the chacma baboon, *Papio porcarius*, questioning Zuckerman's early (1930) statement that there is a regular 28-day cycle, but showing that the cycle is somewhat irregular (29 to 63 days) the average of 20 cycles being 42 days. He also attempted a quantitative description of the changes in the sexual skin by recording four linear measurements by which the rate and extent of the changes may be evaluated. On the basis of later experiments, Gillman (1938) concluded that other factors in addition to estrone withdrawal are responsible for deturgescence and bleeding. Further evidence for the participation of sex hormones in water metabolism was given in a note by Zuckerman, Palmer and Bourne (1939) who point out that estradiol has a specific effect in promoting the movement of water in the rat from muscle and certain viscera to the uterus, vagina and skin. The present paper adds new quantitative observations on the sexual skin and the movements of body water.

MATERIALS AND METHODS. The principal subject of the following report is a female *Papio hamadryas*, the "sacred" baboon, which came to this laboratory in May, 1934. In June, 1934, craniotomy was performed and a small incision was made in the corpus callosum, as a control operation for other studies then in progress. Recovery was prompt and uneventful, and no lasting effects of any kind can be attributed to that procedure.

During periods of continuous observation the animal was kept indoors in a small cage arranged for urine collection. The food supply was varied

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and adequate. Water was usually available, but the animal often filled the water cup with feces. When water intake was to be accurately measured the animal was permitted to drink from a cup held by the observer, water being offered in this manner at least twice a day. The volume of the sexual skin was measured by an immersion method. The baboon, which was quite docile and moderately tame, was held vertically with the hind legs somewhat elevated so that the dependent sexual skin could be dipped into a large vessel of water until it was completely submerged. It was easy to effect complete immersion of the sexual skin with practically none of the rump of the animal. The vessel was supported on the platform of a large springless balance, and the rise in weight was the measure of the volume of water displaced. The error did not exceed 0.2 liter. The weight of the sexual skin was calculated by assuming a specific gravity of 1.05.

The volume of extracellular fluid was estimated as the volume of distribution of injected thiocyanate (Lavietes et al., 1936). A visual colorimeter was used with 1 ml. cups and a Wratten C2 (blue) filter. The amount of thiocyanate bound in the plasma of the baboon was not studied and no correction for possible binding has been applied. Mixing was rapid, as shown by the identity of the thiocyanate concentrations in serum taken at various intervals between 40 and 160 minutes after injection. Sulfanilamide was tried as a means of measuring total body water (Marshall et al., 1937) using the improved method of assay of Marshall and Litchfield (1938), but it was found that the baboon, like man, rapidly conjugates the substance, the combined form appearing both in the urine and in the blood. For that reason the significance of the calculated volume of distribution is of questionable significance and the method was discarded. In its place urea was used, the analytical method being that of Peters and Van Slyke (1932, p. 373). Blood samples were drawn 30, 60 and 90 minutes after the intravenous injection of about 4 grams of urea, and the calculation was based on the blood urea increment extrapolated to the time of injection. Plasma volume was measured with the blue dye, T-1824, using the Evelyn colorimeter with filter 600. Hematocrit determinations were made in Wintrobe tubes using the first blood sample of each experiment. Plasma water content was found by drying 1 ml. at 105°C.

Observations and experiments. The period of most careful observation extended from June 7, 1937, when menstrual bleeding occurred, to July 9, 1938, when it again appeared, making the end of eleven cycles of swelling and shrinking of the sexual skin. In a few instances actual bleeding was not recorded, the cycles being determined by the changes in the sexual skin. The exact length of each period is therefore unknown; the average of the eleven periods is 36 days, which is well within the range

noted by Zuckerman and by Gillman. Since measurements of sexual skin volume were not made in every cycle it cannot be said that the maxi-

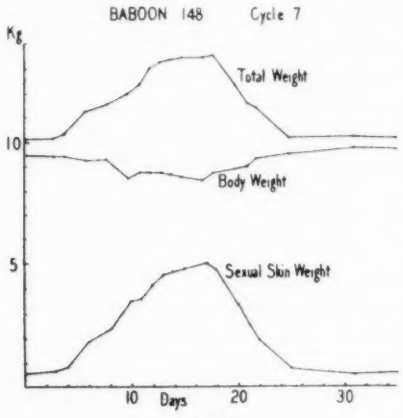

Fig. 1

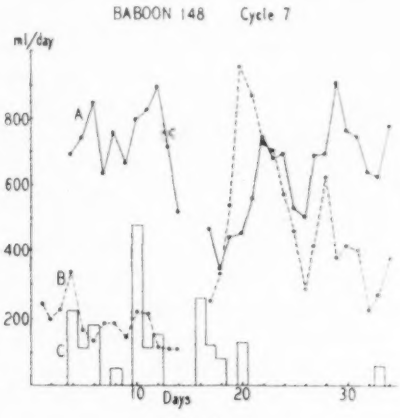

Fig. 2

Fig. 1. Menstrual bleeding on days 1–3 and 35. Nembutal anesthesia on day 15, for measurement of extracellular fluid volume.

Fig. 2. A. Total water intake. B. Urine output. C. Water drunk.

TABLE 1

	ANIMAL 148			ANIMAL 842	
	Weight, kgm.				
	10.4	11.1	12.2	10.0	11.2
Sexual skin:					
Relative swelling, per cent	0	50	100	0	100
Per cent of total weight	2	20	35	2	16
Hematocrit: per cent cells	42	44	47	42	50
Plasma water, mgm./ml.	945	941	949	933	935
Thiocyanate vol., per cent of total weight	27	30	42	27	32
Urea volume, per cent of total weight	60		66		

It was not possible to make all the desired measurements simultaneously, and so the data in table 1 have been assembled from different cycles to illustrate the typical conditions at certain particular stages of the cycle. The second animal, no. 842, was a Guinea baboon, *P. papio*, which did not exhibit so great a swelling of the sexual skin as is seen in *P. hamadryas*.

mal swelling is always the same, but volumes of 3.6, 5.0 and 4.4 liters in cycles 6, 7 and 9 respectively suggest that the maximum is variable.

The time course of the turgescence and deturgescence of the sexual skin was studied by almost daily measurements during cycles 6 and 7, at which

time the major factors of water balance were also recorded. The data for cycle 6 were tabulated in the preliminary report (Clarke, 1938), while those for cycle 7 are summarized in figures 1 and 2. The total water intake includes, with the water drunk, the free water in the food and the calculated water of oxidation, both of the latter being approximated from available data of food composition. The urine output is not corrected for possible losses by evaporation or incomplete cage drainage. In figure 2 the same ordinate scale is used for total weight and computed sexual skin weight. The differences between the two are plotted as the "body" weight in order to emphasize the shift of material from the body to the sexual skin.

Discussion and conclusions. The menstrual cycle, characteristic of the primates, is accompanied in some of them by periodic changes in sexual skin and in other parts of the body. In animals having protruding sexual skin the changes vary tremendously in extent and reach their maximum in some members of the baboon tribe. In the pigtailed macaque, *M. nemestrina*, studied by Krohn and Zuckerman (1937), an increase of 18 per cent in total weight of the animal was found during the sexual swelling, and it was implied that the weight change was confined to the sexual skin. That the situation is not so simple in the baboon is indicated by the results given in the preceding section. The shift of weight to the sexual skin is controlled by so powerful a stimulus that the remainder of the animal loses weight to the extent of at least 1.2 kgm. (e.g., cycles 6 and 7). The blood can be at best a minor contributor. The total blood volume was found to lie between 800 and 900 ml. in a few measurements with T-1824 and the hematocrit. Any alterations in plasma volume at different stages of swelling are not sufficiently great to be established by the data available. The rise in cell fraction (hematocrit) as the swelling proceeds may be a simple replacement of menstrual loss, although transfer of plasma to an extravascular site would give the same result. Since the water content of the plasma, which was accurately measured, was constant, such a transfer would have to be whole plasma including the plasma proteins.

The state of water in the swollen sexual skin is of considerable interest, and a number of observations bearing on the point were made. That it is not a simple edema is shown by 1, the texture and lack of surface wetness on cutting out a biopsy specimen for histological examination, and 2, by the failure to obtain any exudate from a perforated 18-gauge needle deeply inserted for two hours during anesthesia. The source of the mucoid material is unknown, but if it derives directly from the blood there must be such a local alteration in capillary permeability that the colloidal dye, used for plasma volume determination might also escape from the blood vessels. Two biopsy specimens were taken immediately

after plasma volume determinations (sexual skin volumes 0.9 and 2.5 liters) but no blue coloration was visible to the eye, although enough dye had been given to be clearly visible in samples of plasma. It is still held, however, that until this question is answered little weight can be given to plasma volumes thus measured.

Definite conclusions regarding the body water may be drawn from the data on total and extracellular water (volumes of distribution of urea and thiocyanate). The distribution of total weight between body and sexual skin is based on accurate measurements. A slight fall in the extracellular water fraction in the body when the sexual skin is swollen is based on the indication by hematocrit of hemoconcentration, which in the present situation should more or less parallel the extracellular fluid of the body. The animal's relatively increased thirst at this time is further evidence of body dehydration. The intracellular water, calculated by subtracting the thiocyanate volume from the urea volume, is about 3.4 liters when the sexual skin is shrunken, and at the height of the turgescence it has been reduced to about 3.0 liters. This is further proof that the sexual skin swelling is a primary expansion of the extracellular compartment in the specialized tissue, at the expense of cell water and of the interstitial water of the body.

During the phase of swelling the water intake from all sources naturally exceeds the urine output several fold, but promptly at the start of shrinking the urine output is greatly augmented and appetite for fluid water disappears. In cycle 7 the phase of rapid shrinkage occupied days 18 to 24 inclusive, during which time the total water input was 3.84 liters (of which only 0.2 liter was drunk as fluid) while the output by the renal route alone was 4.73 liters. For eleven consecutive days the animal did not drink, and during the 17 days from the start of shrinking to the next menstruation only 0.25 liter of liquid water was imbibed.

When the weight of the sexual skin is plotted against the weight of the body no evidence is found that the swelling and shrinking phases pursue different courses. The data for cycles 6 and 7, thus plotted are scattered fairly closely to a straight line with slope $= -0.2$, which indicates that each kilogram change of sexual skin is accompanied by a 0.2 kgm. change of the body in the opposite direction.

SUMMARY

The sexual skin of the baboon achieves its state of turgescence with little if any change in its cellular volume but a large increase in its interstitial volume. The increase in weight of the organ is in part (20 per cent) contributed by the animal's own body, and the remainder (80 per cent) by the addition of new material from the outside. Thirst and renal func-

tion are closely coördinated with the changes in water balance, in that the animal exhibits thirst and relative oliguria during the rise of the swelling, and the reverse while resorption is going on.

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THE EFFECTS OF DECREASED BLOOD OXYGEN AND INCREASED BLOOD CARBON DIOXIDE ON THE FLOW AND COMPOSITION OF CERVICAL AND CARDIAC LYMPH[1]

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Though the effects of the inhalation of air deficient in oxygen or high in carbon dixoide have been studied in connection with the physiology of many bodily functions, very little has been done with regard to the effects of these agents on the flow and composition of lymph. Landis (1927–1928), by means of his micro-injection technique, studied the effect of complete lack of oxygen on the permeability of the capillary wall to fluid and to the plasma proteins, and was able to show that complete lack of oxygen for 3 minutes brought about a four-fold increase in the rate of capillary filtration and at the same time allowed the plasma proteins to escape into the surrounding tissue spaces to such an extent that the effective osmotic pressure of the plasma was reduced to one-half of the normal value. Landis also reported that complete saturation with carbon dioxide of the fluid bathing the particular tissue with which he was working caused only a slight increase in the rate of capillary filtration and allowed the escape of none of the plasma proteins, while half saturation of the bathing fluid with carbon dioxide caused no change at all in the rate of formation of capillary filtrate.

Gesell (1928) reported that low oxygen and high carbon dioxide increased the flow of thoracic duct lymph, with a subsequent retardation during recovery. He stated, also, that as the flow increased the lymph became increasingly turbid, and suggested that this turbidity indicated increased permeability of the vascular membranes involved. He did not state, however, the effective concentrations of these agents. Though not directly related to the problem of lymph production, the studies of Campbell (1929), who compared the pathological effects of prolonged exposure to carbon monoxide and to very low oxygen tensions, suggest the possible effects of these agents on the production of capillary filtrate and subsequently of lymph. As a result of his studies, he reported that prolonged exposure to carbon monoxide and to low oxygen tensions caused among

[1] This investigation was aided by the Miriam Smith Rand Fund.

other things the production of edema and dropsy, undoubtedly the result of increased capillary filtration, which would favor increased lymph production.

McMichael and Morris (1936) attempted to determine the effect of acute oxygen lack on capillary permeability in man by making measurements of arm volume, following the method of Smirk (1935–1936). They observed that the inhalation of gas mixtures containing concentrations of oxygen as low as 9.5 per cent was without effect on the rate of swelling of the human arm, and that high percentages of carbon dioxide in the inspired air were also without effect on the rate of swelling, even when combined with low oxygen percentages. Unfortunately, the duration of these experiments was not stated. Finally, Saslow (1938) reported that solutions of 3 per cent acacia and 19 to 24 per cent ox red cells in Ringer's solution could be perfused through frogs for periods up to 6 hours without the appearance of microscopically detectable edema in the web. He stated that the effectiveness of this solution in preventing edema formation appeared to be due to its high content of available oxygen.

The present work consists of the results of twenty experiments performed on dogs in an attempt to determine the effects of the inspiration of gas mixtures low in oxygen and of underventilation upon the production and composition of cervical and cardiac lymph.

EXPERIMENTAL TECHNIQUE. All of the experiments were performed on healthy young adult dogs under nembutal anesthesia (40 mgm. per kgm. intravenously). The procedure was essentially that described by McCarrell (1939) for the continuous collection of cervical lymph, this preparation being known by us as the "nodding dog."

While the preparation was in progress, an infusion of 20 cc. per kgm. of warm Ringer's solution was given to ensure thorough hydration of the animal. Arterial blood pressure was recorded by the usual mercury manometer. The animal's temperature was measured at various intervals by means of a rectal thermometer.

Except when the animal was made to breathe room air, gas mixtures of known concentration were made up in pressure cylinders and delivered to an 80-litre spirometer, from which the gas was drawn into a respiration pump designed so that any given volume of air could be delivered into the lungs at the rate of 14 inspirations per minute. Rebreathing experiments were performed by establishing a closed circulation between the spirometer and the animal, soda-lime being used for the removal of carbon dioxide. In this manner the oxygen saturation of the blood could easily be determined at the exact time that the lymph flow began to change.

Arterial blood samples were collected from the femoral artery into blood tubes in the usual manner, and were analyzed in the Van Slyke gas analyzer for oxygen and carbon dioxide. Gas samples from the spirometer were analyzed in the Haldane gas analyzer.

At the beginning of each experiment 1 cc. of 1 per cent curare diluted to 20 cc. with Ringer's solution was injected intravenously, in order to eliminate the forced inspiratory movements which always occur with the inspiration of low oxygen or high carbon dioxide mixtures. Such forced inspirations, if allowed to persist, would produce wide irregularities in the flow of lymph. Both the nembutal and the curare were repeated during the experiment in small doses as required.

RESULTS. *Exposure to low oxygen mixtures of known concentration.* In order first to determine the effect of low oxygen pressures upon the flow of cervical lymph, the animals used in the first few experiments were

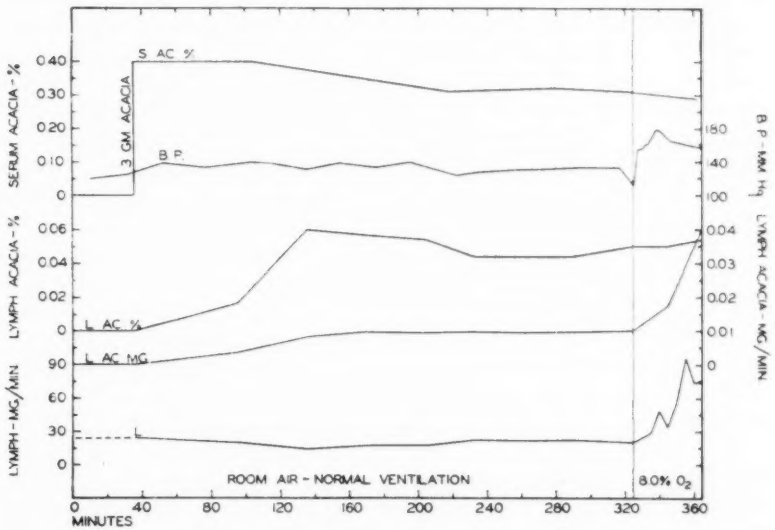

Fig. 1. Normal cervical lymph flow, followed by exposure to 8.0 per cent oxygen. Passage of intravenously injected acacia from blood to lymph. Increased capillary permeability with low oxygen shown by increased output of lymph acacia.

exposed to oxygen-nitrogen mixtures containing from 8.0 to 10.5 per cent of oxygen, though in a few cases mixtures containing as little as 4.0 per cent were used.

The experiment illustrated in figure 1, though performed for a different purpose, demonstrates very clearly the strikingly uniform flow of cervical lymph which can be obtained by this so-called "nodding dog" technique. Over a period of 5 hours and 25 minutes this dog was made to inspire adequate volumes of room air from the respiration pump. Throughout this period the average lymph flow was 19.9 mgm. per minute, the average deviation being only 2.4 mgm. per minute. At the end of this long control period, the animal was exposed to a gas mixture containing 8.0 per cent

oxygen. Immediately the flow of lymph began to increase, and continued to do so for 30 minutes when the peak flow was reached. This peak amounted to 4.8 times the normal flow. Though the administration of the low oxygen was continued, the lymph flow decreased during the next 10 minutes, following which the experiment was terminated.

Figure 2 illustrates a typical experiment in which the blood gases, arterial pressure, and blood and lymph proteins were determined as well as the

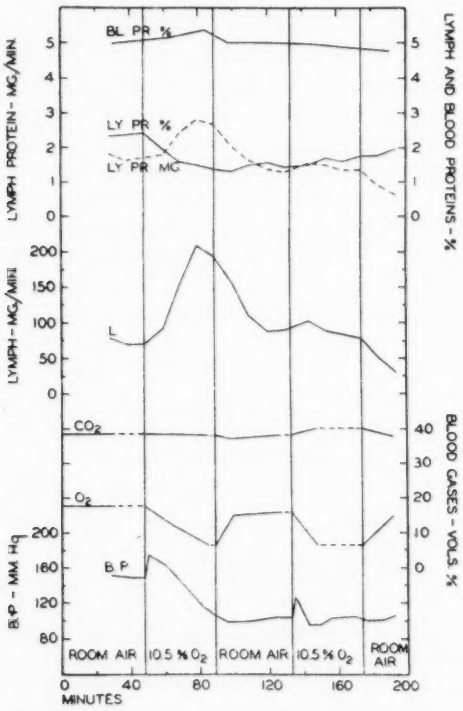

Fig. 2. Cervical lymph flow, lymph and serum proteins, blood gases, and arterial blood pressure of a dog alternately exposed to room air and 10.5 per cent oxygen.

lymph flow. During a control period lasting for about 50 minutes, the animal received room air from the respiration pump and, as the illustration shows, everything remained quite constant. At the end of this period the animal was shifted to a mixture containing 10.5 per cent oxygen, exposure to this mixture continuing for the next 41 minutes. During the first 2 minutes of low oxygen the arterial blood pressure rose 27 mm. of mercury, and then fell gradually and steadily until the end of the period, when it had reached a value of 100 mm. of mercury. The control blood pressure

was 150 mm. of mercury. The blood oxygen began to decrease immediately from its control value of 17.7 volumes per cent, and continued to decrease until at the end of the period it had reached 6.5 volumes per cent. The blood carbon dioxide remained practically constant throughout the entire experiment. Immediately upon the shift to 10.5 per cent oxygen, the lymph flow began to increase from its control level of 71 mgm. per minute. This increase continued until at the end of 31 minutes it had reached 208 mgm. per minute, or approximately 3 times the control value. Though the low oxygen continued for another 10 minutes, this peak flow of lymph was not maintained, but fell during this time approximately 15 mgm. per minute.

The animal was then shifted back to room air. The blood oxygen returned to approximately the normal value, and the lymph flow returned gradually to within 16 mgm. per minute of the control level. The flow remained constant at this figure for 13 minutes, when the animal was shifted again to the 10.5 per cent oxygen mixture. The arterial blood pressure again showed the short sharp rise which is characteristic of exposure to low oxygen. This rise subsided quickly and the blood pressure remained fairly constant for the duration of the experiment. The blood oxygen fell, as during the previous exposure, to 6.5 volumes per cent. During the first 11 minutes of this second exposure to low oxygen, the lymph flow increased only 17 mgm. per minute, or 1.2 times the normal value. The low oxygen was continued for 30 more minutes, during which time the lymph flow gradually subsided to approximately the control level. At this point the animal was shifted back to room air, and for 20 minutes the flow continued to fall until it was 40 mgm. per minute below the control level.

A total of 9 animals were similarly exposed to low oxygen. In each of these animals lymph flow was increased. Not all, however, responded to this treatment exactly as did the animal of figure 2. The range of increases was from 1.2 to 4.8 times the control flow. There appeared to be differences in the susceptibility of different animals to this treatment. For example, one dog reached a peak flow of 2.6 times the normal in 10 minutes; while another reached a peak flow of only 1.4 times in 97 minutes. A few of these 9 animals were exposed to low oxygen a number of times with an adequate interval of room air between exposures. The results of such repeated exposures can be summarized as follows:

1. After a short initial exposure to low oxygen a second exposure to the same concentration of oxygen will, in the majority of cases, bring about a second increase in flow which will invariably be less than the initial increase. Figure 2 shows the usual effect of such a second exposure. In some cases, however, the second exposure either will cause no increase at all or may be responsible for a decreased flow.

2. Following a short initial exposure, a second exposure to a gas mixture containing less oxygen than that of the first will in some animals produce a flow nearly equal to and occasionally greater than the initial increase. Here again, however, some animals will not respond or will show a decrease.

3. Following a long exposure (over 40 min.) to low oxygen, it becomes increasingly difficult to produce a secondary increase in lymph flow. In some cases following long exposure, mixtures containing as little as 4.0 per cent oxygen had little or no effect on the flow other than to cause some retardation.

It has already been mentioned that once the maximum flow has been reached, continued exposure to the low oxygen mixture does not cause this high rate of flow to persist. Even though the animal continues to breathe the low oxygen mixture for many minutes beyond the peak, the flow will fall considerably below the maximum, in many cases returning to or nearly to the control level within the period of exposure. In one experiment it was found that the flow of thoracic duct lymph increased when the animal inspired low oxygen mixtures, just as the cervical flow increased. Gesell (1928) reported a similar finding. Certainly, then, exposure to low oxygen tensions must cause similar increases in all of the lymphatic channels of the body. A two- or three-fold increase in the lymph flow in the entire lymphatic system must represent a tremendous loss of fluid from the circulating blood in a very short period of time. Since this is undoubtedly true, the consequent increase in the colloid osmotic pressure of the blood serum (Landis, 1927–1928) would be sufficient to prevent further loss of fluid, even in the face of the damage to the vascular membranes brought about by the low oxygen saturation of the blood. It is easy to understand, therefore, that further exposure of the animal to low oxygen will have little or no effect upon lymph production, and in many cases will result in a diminished flow.

In a few experiments lymph flow remained somewhat higher than the control level, even after the animal had been shifted back to room air for a considerable length of time. Such a finding might be taken to indicate that some permanent damage had been done to the capillaries, and even though the colloid osmotic pressure was higher than normal the damage may have been great enough to permit a continuous leaking of fluid and protein from them.

Underventilation and exposure to high carbon dioxide mixtures. Early in this series of experiments 3 dogs were exposed to carbon dioxide-oxygen-nitrogen mixtures containing 8.9, 5.1 and 17.0 per cent of carbon dioxide, respectively. The arterial blood pressure showed a sharp initial rise, after which it returned approximately to the normal value where it remained fairly constant. The blood oxygen showed an average increase

of 1.4 volumes per cent, while the carbon dioxide increased 2.6, *13.7* and 15.2 volumes per cent, respectively. In each case there was an almost immediate increase in lymph flow, amounting in the respective animals to 2.8, *1.7* and 2.1 times the normal rate.

Figure 3 is an illustration of the findings from the second of these experiments, the exact values for which appear in italics in the preceding

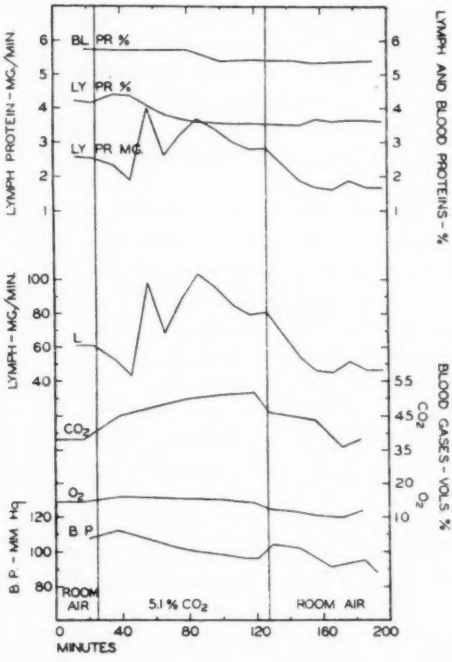

Fig. 3

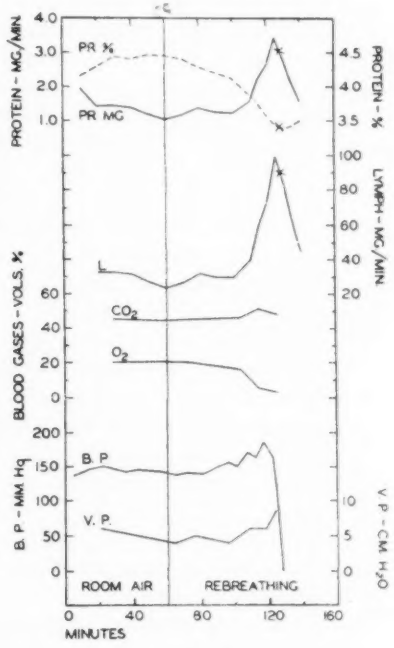

Fig. 4

Fig. 3. Cervical lymph flow, lymph and serum proteins, blood gases, and arterial blood pressure of a dog exposed to 5.1 per cent carbon dioxide.

Fig. 4. Cervical lymph flow, lymph protein, blood gases, and arterial and venous blood pressures of a dog rebreathing from an 80-litre spirometer until circulatory collapse.

paragraph. With regard to lymph flow the results of these three experiments are not unlike those of the low oxygen experiments.

At the beginning of each of the 20 experiments of this series, a control period was run during which the animal breathed room air delivered by the respiration pump. In adjusting the pump so that the animal received adequate ventilation, it was discovered that the blood carbon dioxide could

be increased without significantly lowering the oxygen content by the simple expedient of decreasing the volume of air delivered. Five animals were subjected to this treatment. The output of the pump was first adjusted so that the air delivered to the animal was adequate to maintain the normal oxygen and carbon dioxide content of the blood. This was determined by analyzing arterial blood samples. After a short preliminary period the inspiratory volume was decreased anywhere from 17 to 58 per cent. The blood oxygen showed only insignificant changes, the greatest decrease observed being only 3.7 volumes per cent. In every instance the carbon dioxide content of the blood increased, the smallest increase observed being 2.0 volumes per cent, the largest 8.9 volumes per cent. As a result of this treatment each animal responded with an increased production of cervical lymph. These increases ranged from 1.3 to 3.0 times the normal flow.

Here again there appeared to be differences in the susceptibility of the animals to the increased carbon dioxide. In one instance it required only 25 minutes to increase the flow 3 times, while in another it required 40 minutes to attain an increase of 2.4 times, and in still another 30 minutes were required to bring about an increase of only 1.3 times.

Following this treatment, the animals were given normal ventilation for sufficient time to reduce their blood carbon dioxide to the control level, after which they were exposed to low oxygen mixtures. In each case there was a second increase in the lymph flow which was equal to or greater than the increase resulting from the underventilation. This observation would seem to indicate that the damage caused by increasing the carbon dioxide load of the blood was less extensive and of much shorter duration than that caused by decreasing the oxygen saturation, for, as already pointed out, it is practically impossible to increase the flow of lymph after an initial treatment with low oxygen.

Having observed the effect of increased carbon dioxide, there appeared the possibility that the increased lymph production resulting from a deficient oxygen supply was not due alone to anoxemia but was the combined result of decreased oxygen and increased carbon dioxide. Careful checks of the blood gas analyses of the low oxygen experiments showed that in only one case had the carbon dioxide content of the blood risen more than 1.0 volume per cent during the period of the increased flow. All of the other experiments showed that the carbon dioxide had remained at the control level or had fallen slightly below. It is safe, therefore, to say that anoxemia alone is capable of producing the changes observed following exposure to low oxygen.

Rebreathing experiments. In order to determine exactly the degree of oxygen desaturation necessary to increase lymph production, 6 rebreathing experiments were performed. By making use of this procedure it was

possible to reduce the blood oxygen gradually over a long period of time. By repeatedly analyzing the blood gases, the desaturation curve was drawn and compared with the lymph production curve. Figure 4 illustrates the results of one of these experiments. Careful analysis of the data revealed that the flow of lymph began to increase when the oxygen saturation reached 70.7 per cent of normal. As the rebreathing continued the blood oxygen saturation decreased more rapidly, being accompanied by an increasingly rapid lymph production. When the slope of the lymph curve became steepest, the saturation of the blood was only 46.3 per cent. The maximum flow was 3.3 times normal, and at this time the oxygen saturation was only 14.6 per cent. Four minutes after attaining the maximum flow, the oxygen supply was so completely diminished that the animal died. It is interesting to compare the oxygen saturation of this blood when lymph flow began to rise, with the chart of oxygen saturation in relation

TABLE 1

Relation of cervical lymph flow to blood oxygen saturation and altitude

EXPERIMENT	INCREASED FLOW		STEEPEST FLOW		MAXIMUM FLOW	
	O_2 saturation	Altitude	O_2 saturation	Altitude	O_2 saturation	Increase over normal
	per cent	*feet*	*per cent*	*feet*	*per cent*	*times*
1	70.7	18,500	46.3	>20,000	14.6	3.3
2	76.7	16,500	62.5	>20,000	17.0	3.2
3	82.6	14,000	75.9	17,000	69.0	3.9
4	69.8	19,000	40.7	>20,000	12.5	1.8
5	74.5	17,500	45.2	>20,000	36.0	1.6
6	75.5	17,000	44.3	>20,000	13.0	2.6
Average...	75.0	17,000	52.5	>20,000	27.0	2.7

to altitude published by Dill, Bock, Edwards and Kennedy (1936). According to their figures, this particular animal had reached an altitude equivalent to 18,500 feet.

Table 1 shows the results of the 6 rebreathing experiments. Inasmuch as 14,000 feet is generally considered to be the altitude at which most mountain climbers and other high altitude workers are first seriously affected by oxygen lack, it is interesting to note that the average saturation of these 6 animals was 75.0 per cent, which is roughly equivalent to an altitude of 17,000 feet. Since the susceptibility of these dogs varied widely, the range being approximately from 14,000 to 19,000 feet, it should be quite safe to say that the critical saturation with regard to lymph flow was roughly equal to 14,000 feet also. To continue this analogy, it is interesting to note that the average saturation when the slope of the flow curves was greatest is 52.5 per cent, which is equivalent to an altitude greater

than 20,000 feet, beyond which it is very difficult for man to venture without the aid of oxygen.

The effect of low oxygen on cardiac lymph flow. One experiment was performed in which both the cervical and cardiac lymph flows were observed during exposure to low oxygen mixtures. The cannulation of the cardiac lymphatic duct has been described by Drinker, Warren, Maurer and McCarrell (1940), and except for this addition, the procedure was no different from that used in the other low oxygen experiments. Figure 5

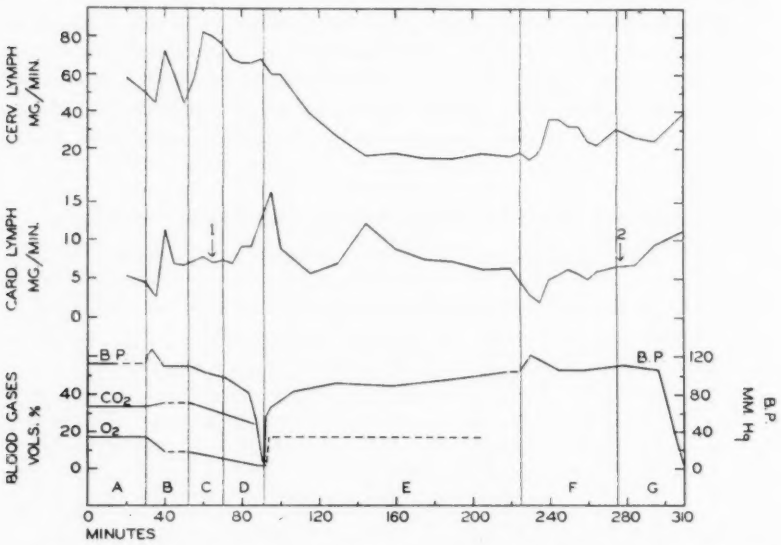

Fig. 5. Cardiac and cervical lymph flows, blood gases, and arterial blood pressure of a dog during exposures to low oxygen and to 100 per cent oxygen. *A*, room air control; *B*, 8.0 per cent oxygen; *C*, 6.0 per cent oxygen; *D*, 4.5 per cent oxygen; *E*, 100 per cent oxygen; *F*, 6.0 per cent oxygen; *G*, 4.5 per cent oxygen. At arrow *1*, cardiac lymph became slightly bloody and remained so until, at arrow *2*, it became very bloody.

illustrates the results. In general the flow of cardiac lymph followed much the same course as that of the cervical lymph, showing an initial increase of 2.5 times on exposure to 8.0 per cent oxygen and a four-fold increase with 4.5 per cent oxygen. There was observed, however, one striking difference. During period *D* of figure 5, the animal was exposed to 4.5 per cent oxygen for 21 minutes, causing the arterial blood pressure to fall to only 10 mm. of mercury. At this point the animal was shifted to 100 per cent oxygen, which was continued for 2 hours and 14 minutes. Section *E* illustrates the flows during this time, and it is during this period that

the difference is noted. The flow of cardiac lymph maintained a minimum rate which was never less than 1.4 times the control level, while the cervical flow fell considerably below the control level and remained there. It seems possible that the decreased oxygen supply had a more damaging influence on the capillaries of the heart than those of the region drained by the cervical ducts. This is substantiated not only by the increased flow maintained throughout section *E*, but also by the fact that at arrow *1*, the cardiac lymph became slightly bloody and remained so throughout the experiment. Further exposure to low oxygen (sections *F* and *G*) caused the lymph flows to increase again. During this time (arrow *2*) the cardiac lymph became more bloody than before, while the cervical lymph still showed no change in color. The appearance of red cells and hemoglobin in the cardiac lymph must certainly denote a high degree of cardiac capillary damage.

Lymph protein. Total protein was determined by means of the Zeiss dipping refractometer calibrated against known samples of dog serum and lymph, and frequently checked by micro-Kjeldahl determinations. The data are in accord with findings reported many times from this laboratory. As long as lymph flow remains constant, the percentage protein in the lymph also remains constant; and when lymph flow increases, the percentage of protein decreases, returning to the original value when the flow returns to normal. However, when lymph protein is calculated in milligrams per minute (lymph flow in milligrams per minute × per cent protein), it is found that the output of protein varies directly with the rate of lymph flow. Figures 2, 3, 4, 6 and 7 clearly illustrate this relationship.

In contrast with the statement of Landis (1927–1928) that carbon dioxide allowed the escape of none of the plasma proteins into the capillary filtrate, the present work shows that both increased carbon dioxide and decreased oxygen have similar and equal effects upon the protein content of cervical lymph.

This augmented output of protein in the lymph during exposure to low oxygen or high carbon dioxide is believed to indicate that the permeability of the capillary walls is greatly increased. Further indication of increased permeability is shown by the fact that while the lymph flow and lymph protein output are increasing, there is a simultaneous decrease in the concentration of the serum proteins. This is clearly illustrated in figures 2 and 3.

Lymph albumin and globulin. Drinker, Warren, Maurer and McCarrell (1940) showed that the albumin/globulin ratio of cardiac lymph was, in most of their experiments, approximately equal to the ratio in the blood of the same animal. In the present work the same was found to be true of the albumin/globulin ratios of cervical lymph. To determine the effect of exposure to low oxygen or high carbon dioxide and the resulting increase

in lymph flow upon the albumin/globulin ratio of cervical lymph, these protein fractions were determined at frequent intervals throughout 6 experiments. Albumin was determined by precipitating the globulin with 22.5 per cent sodium sulphate, filtering off the precipitate, and making nitrogen determinations on the filtrate by micro-Kjeldahl analysis, a modified Pregl apparatus being used for the distillation. Globulin was determined by difference.

In each of these experiments it was found that the albumin/globulin ratio, calculated from the output of each in milligrams per minute, was

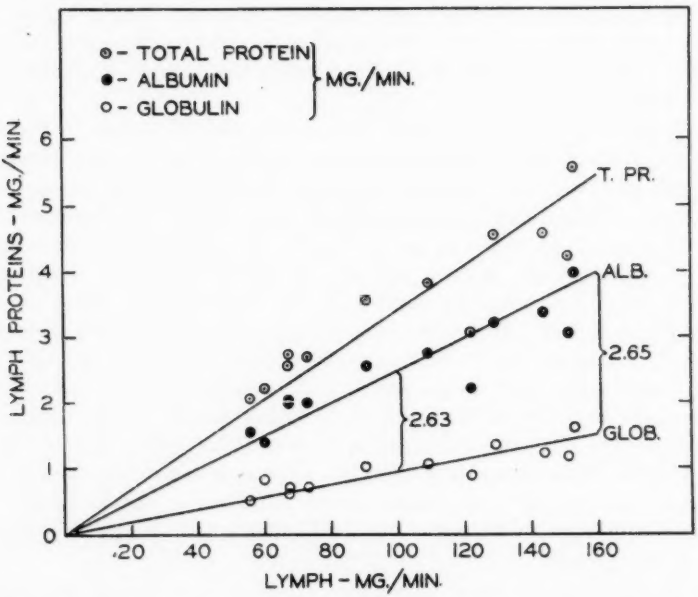

Fig. 6. The relation of cervical lymph protein, albumin, and globulin in milligrams per minute to the rate of lymph flow in milligrams per minute. Flow was increased in this dog, first by underventilation and later by rebreathing.

constant regardless of the rate of lymph flow. Figure 6 shows this constant relationship in one experiment in which lymph flow was made to vary between 56 and 154 mgm. per minute.

Acacia experiments. In 6 of the present experiments 3 grams of gum acacia (50 cc. of a 6 per cent solution in saline) were injected intravenously, and for the duration of the experiments samples of blood and lymph were analyzed for acacia as described by Maurer, Warren and Drinker (1940). Maurer *et al.* have stated that it is their belief that the protein of extra-

cellular fluids is derived from the protein of the circulating blood. To substantiate this belief, they quoted experiments in which the passage of acacia and horse serum was followed from the blood stream into the pericardial and peritoneal fluids of dogs. Drinker, Warren, Maurer and McCarrell (1940) utilized the passage of these foreign substances from the blood stream into cardiac lymph to demonstrate the permeability of the cardiac blood capillaries. The present acacia experiments were performed not only to shed further light upon the source of lymph protein, but to substantiate the belief that the permeability of blood capillaries is increased as a result of decreased blood oxygen or increased blood carbon dioxide.

In the experiment illustrated in figure 1, the passage of acacia from the blood stream into the cervical lymph was demonstrated. The amount of acacia appearing in the lymph reached a maximum value of 0.01 mgm. per minute after 2 hours and 17 minutes, and remained at this level for another 3 hours and 8 minutes. It is significant that until this steady level of lymph acacia had been reached, the acacia in the blood stream decreased steadily and then it, too, remained constant. At the end of this long control period, anoxemia was induced by exposing the animal to 8.0 per cent oxygen. Coincident with the increased lymph flow, there was an immediate and sharp rise in the output of acacia into the lymph to 0.04 mgm. per minute, the percentage of lymph acacia remaining practically constant. In contrast to this increased output of acacia into the lymph there was a further decrease in the concentration of serum acacia.

Figure 7 illustrates another of these acacia experiments in which lymph protein and lymph acacia were followed simultaneously. Three distinct increases in lymph flow were produced, the first and third (sections *B* and *F*) by exposure to 8.0 and 7.0 per cent oxygen, respectively, and the second (section *D*) by a 58 per cent reduction of the inspiratory volume. The acacia was administered to this animal 49 minutes before the data shown in figure 7 were obtained. During that time the output of lymph acacia had almost reached a steady state. As in all the other experiments the percentage of lymph protein decreased coincident with the increased rate of flow, while the output of protein in milligrams per minute increased, both of these values approaching normal as the rate of flow decreased. It is significant that the acacia curves of this figure, both for percentage and for output in milligrams per minute, very nearly parallel the protein curves.

These experiments not only demonstrate that the blood capillaries are normally permeable to acacia and to protein, but show also that anoxemia and high blood carbon dioxide can greatly increase this permeability. Also, having observed that coincident with an increased rate of lymph flow there is an increase in the output of protein and acacia into the lymph (figs. 1, 2, 3, 4, 6 and 7) and a decreased concentration of these substances

in the blood stream (figs. 1, 2 and 3), there can be little doubt that the normal protein constituents of lymph are derived from the protein of the circulating blood.

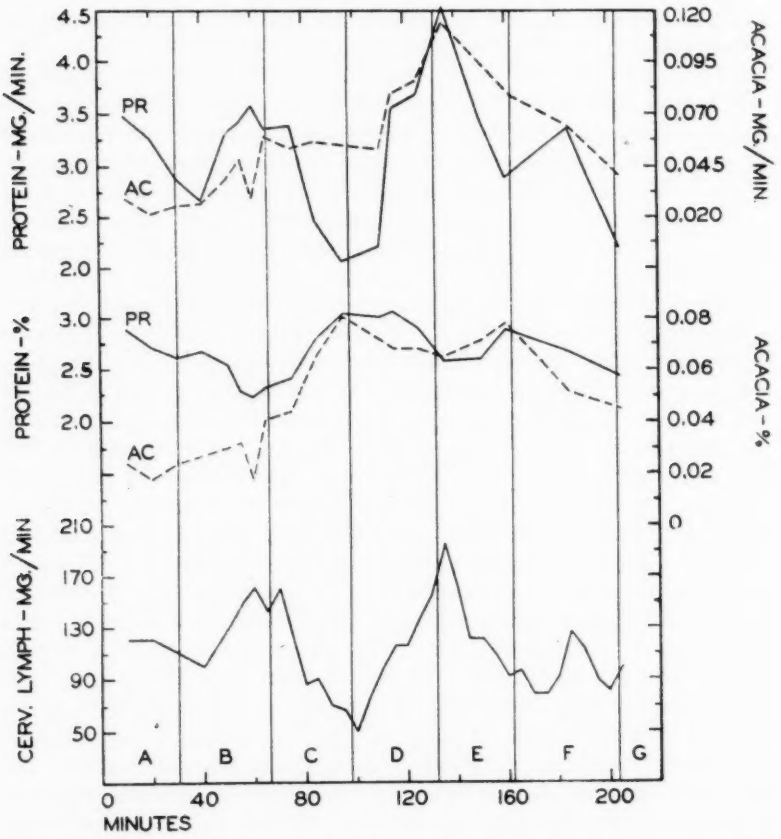

Fig. 7. Per cent lymph protein and lymph acacia, output of lymph protein and lymph acacia in milligrams per minute, and cervical lymph flow of a dog during exposure to low oxygen and during underventilation. *A*, room air control; *B*, 8.0 per cent oxygen; *C*, room air; *D*, inspiratory volume decreased 58 per cent; *E*, normal ventilation; *F*, 7.0 per cent oxygen; *G*, room air. Three grams of acacia (50 cc. of a 6.0 per cent solution) were injected intravenously 49 minutes prior to the beginning of section *A*.

Discussion. In each of the 20 experiments reported here, the arterial blood pressure was recorded at frequent intervals. Figures 1, 2, 3, 4 and 5 illustrate very clearly the effects of exposure to low oxygen and to high

carbon dioxide upon this pressure. The general effect is that of a short and rapid increase averaging 30 mm. of mercury and in these experiments never exceeding 51 mm. of mercury. In 6 of these experiments the venous blood pressure was measured in the external jugular vein. Here, the effect of the exposures ranged from a fall of 2.6 cm. of water to a rise of 5.4 cm. of water. Figure 4 shows the venous pressure recordings of one of these experiments. The question may arise as to whether these relatively small increases in arterial and venous blood pressures may have themselves been responsible for the increases in lymph flow that were observed. Haynes (1932) has shown that the arterial pressure of dogs was increased 3 times before there appeared any increase in the flow of subcutaneous lymph. McMaster and Hudack (1932) have reported that the permeability of the capillaries of the frog's web was increased only when the venous pressure approximated that in the small arteries. Krogh, Landis and Turner (1932) have stated that fluid accumulated in the tissue spaces of the human arm only when the venous pressure exceeded 15 to 20 cm. of water. Since the greatest increase in arterial pressure recorded was only 1.4 times the normal and since the highest venous pressure observed was 11.3 cm. of water, it seems very improbable that the pressure changes observed in these experiments were in any way responsible for the increased production of lymph accompanying exposure to low oxygen or high carbon dioxide. It is also significant that the venous pressure rise does not occur until some minutes after a considerable increase in lymph flow has been attained.

Several of the animals were exposed to low oxygen or made to rebreathe until the circulation had almost completely collapsed. At this point some were allowed to die while others were shifted to 100 per cent oxygen for varying lengths of time. Immediately the blood pressure rose to or nearly to the normal level. Though all of these animals had been exposed to low oxygen longer than 40 minutes (48 to 100 min.), it is interesting to note the varied responses in lymph flow after the shift to 100 per cent oxygen. Two of the animals were exposed to 100 per cent oxygen for 33 and 13 minutes, respectively, the flow of the first remaining much higher than normal, the second actually showing an increase of 59 mgm. of lymph per minute. The cardiac lymph flow of a third animal (shown in fig. 5) remained higher than normal and even showed transient increases during 2 hours and 14 minutes of 100 per cent oxygen. In contrast to these results, 2 other animals were given this treatment for 60 and 70 minutes, respectively, the flow of the first falling below normal, that of the second falling just to normal, while the cervical flow of the animal of figure 5 also fell considerably below normal. From so little and such widely varying data it is impossible to judge what the real effect of the 100 per cent oxygen was. Perhaps the 3 decreased flows observed were simply the natural result of

the increased osmotic pressure which followed the great loss of fluid from the circulating blood. There can be no doubt that 100 per cent oxygen did not have the beneficial effect upon the cardiac capillaries that it appeared to exert upon the capillaries of the region drained by the cervical ducts in the experiment of figure 5. The real significance of such treatment cannot be determined until similar experiments are performed during which the animal is exposed to pure oxygen for periods of many hours.

The question of acclimatization to the low oxygen tensions of high altitude has intrigued many physiologists. Talbott and Dill (1936), in describing their observations of persons living at the altitude of 17,500 feet in the mountains of northern Chile, report that these people, though constantly exposed to an atmosphere with a decreased oxygen pressure, appear to be normal in most respects except for the fact that the average arterial oxygen saturation of those observed was 75 per cent. It is interesting to note that the arterial saturation of these people reported by Talbott and Dill is the same as the average saturation at which lymph flow began to increase in the present experiments. Perhaps these dogs would have become acclimatized to low oxygen if exposed for sufficiently long periods of time, and if such were the case, it is possible that increased lymph flows would not have been observed. One of the present experiments is perhaps significant in this respect. This experiment was performed upon a dog weighing 11 kgm., from which had been drawn approximately 1500 cc. or more of blood over a period of about 4 weeks. At the time of the experiment the animal appeared quite normal, though upon analysis of her blood there were found only 8.7 volumes per cent of oxygen. This animal rebreathed over a period of 2 hours, until the blood oxygen had dropped to 4.8 volumes per cent. During the first 40 minutes of the rebreathing, lymph flow decreased 8 mgm. per minute; during the next 50 minutes, the flow returned to the original value; and from that time on, during the next 30 minutes, the flow decreased steadily to a level of 16 mgm. per minute below the control. It is remarkable that this dog, whose arterial saturation was less than 50 per cent, appeared to be completely normal. It is not impossible that this animal had become acclimatized to this greatly decreased arterial saturation, and therefore did not respond on exposure to air low in oxygen as did the 19 other dogs of this series of experiments.

Interesting also in relation to the present work is the report of Graybiel, Missiuro, Dill and Edwards (1937) on experimental asphyxiation in cardiac patients. They conclude "that many cardiac patients are endangered when the oxygen of the inspired air falls to 12 per cent, which would correspond to an elevation of 14,500 feet. The untoward effects observed may be due to the general unfitness which is so often associated with heart disease or due more directly to embarrassment of the heart itself." The

experiment of figure 5 is perhaps significant in this respect, for here was seen in the heart of a normal dog a four-fold increase in the production of cardiac lymph as well as the appearance of erythrocytes in this fluid after a relatively short exposure to low oxygen. If exposure to low oxygen can produce such long-lasting changes in the lymph production of a normal heart, it is not hard to understand that the heart of a cardiac patient might be embarrassed under similar circumstances.

Perhaps, too, some of the symptoms that appear on rapid ascents to altitudes of 14,000 feet or over, or during the exposure of unacclimatized individuals to low oxygen tensions, may in part be due to the great loss of fluid from the circulating blood, observed in the form of increased lymph production, and to the accompanying increase in the colloid osmotic pressure of the blood serum.

The author takes this opportunity to thank Miss Anne C. Messer for technical assistance in gas analysis.

SUMMARY

Experiments are reported in which dogs were exposed to low oxygen tensions and to increased tensions of carbon dioxide. Such treatment results in either case in the increased production of cervical lymph.

Following initial exposure to low oxygen, it becomes increasingly difficult to produce secondary increases in lymph flow by further exposures. However, initial exposure to increased carbon dioxide has little or no effect upon the production of secondary increases in lymph flow by exposure to low oxygen.

Rebreathing experiments showed that increased lymph production began when the arterial oxygen saturation reached 75 per cent, which is equivalent to an altitude of 17,000 feet, and that the production of lymph became greatest when the arterial saturation reached 52.5 per cent, which is equivalent to an altitude greater than 20,000 feet.

It was shown that the damage to cardiac blood capillaries was significant as reflected by greatly increased flow of cardiac lymph, the persistence of this increased flow even after long exposure to pure oxygen, and the appearance of erythrocytes in the lymph.

Without exception the percentage of protein in the lymph decreased with increased flow and returned to normal when the flow subsided. The passage of protein from the blood capillaries to the lymph, calculated in milligrams per minute, increased as flow increased and decreased as flow subsided. Coincident with increased output of lymph protein there was a decrease in the concentration of serum protein.

Acacia, injected intravenously, regularly appeared in the lymph. Equilibrium between serum acacia and lymph acacia was attained in from 40 minutes to 2 hours. With increased lymph flow, the course of lymph and serum acacia paralleled that of the lymph and serum proteins.

It was shown that the ratio between lymph albumin and lymph globulin remains constant with all rates of lymph flow.

The increased passage of protein and acacia from the blood stream into the lymph is believed to indicate that low blood oxygen and increased blood carbon dioxide result in increased capillary permeability with loss of fluid and protein from the circulating blood. This loss of fluid leads to increased colloid osmotic pressure of the blood serum.

The possible implications of this increased lymph flow are discussed.

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A QUANTITATIVE STUDY OF ACID IN THE INTESTINE AS A STIMULUS FOR THE PANCREAS

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The fact that acid in the intestine causes the pancreas to secrete has been known for almost half a century (Bekker, 1893). Literature dealing with the subject has been reviewed by Pavlov (1910), Babkin (1914), and Ivy (1930). Although acid was one of the first pancreatic secretagogues discovered, its physiological significance is still uncertain. We do not know how much acid must be present in the intestine to stimulate the pancreas nor how acid the intestinal contents may become during digestion.

An attempt has been made by one of us (Thomas, 1940) to determine the maximal acidity of the intestinal contents of the dog during the digestion of raw meat. Our purpose in the present investigation was to ascertain the minimal acidity that would suffice to stimulate the pancreas. By determining the acid threshold (in pH units) for comparison with the acidity observed in the intestine, we hope to provide a basis for estimating the significance of acid as a stimulus for pancreatic secretion during normal digestion.

METHOD. The method that we used has not previously been applied to the study of pancreatic function although a similar but less convenient procedure was described by Tuckerman (1883). Dogs were provided with gastric and duodenal fistulae fitted with large cannulae (minimal internal diameter $\frac{5}{8}$ in.) as described previously (Thomas and Crider, 1934). For these studies the duodenal fistula was placed at the level of the main pancreatic duct in the duodenal wall directly opposite the opening of the duct.

Instead of attempting to cannulate the duct through the fistula-tube as Tuckerman did, we collected the secretion during the periods of observation through a funnel-shaped rubber cup which was held lightly against the duodenal mucosa surrounding the duct by means of a conical coiled spring attached to the cannula. The arrangement is illustrated in figure 1. The wall of the cup was deeply grooved on the outside to provide drainage for extraneous fluids. Control experiments on an animal with the duodenal fistula below the level of the duct proved that contamination of the secretion with succus entericus was quantitatively unimportant.

Three dogs were used, in one of which the common bile duct was transplanted into the stomach and the accessory pancreatic duct ligated. Observations were made when the stomach and small intestine were empty, 18 to 24 hours after a small meal of selected lean meat.

The animal was supported in a standing position on a table by means of a comfortable muslin harness suspended from a horizontal rod. The secretion dripped into a funnel which was connected through a displacement bulb to a drop recorder of the Wesson (1933) type. This apparatus was filled with distilled water and leveled so that addition of fluid in the funnel caused an equal volume of water to drop from the pipette.

To prevent fasting gastric secretion (or bile in the animal with the transplanted duct) from entering the duodenum in the course of an experiment, the antrum and body of the stomach were drained through two tubes via the gastric fistula. The antral tube was attached to a suction apparatus which maintained a negative pressure of 20 to 30 cm. of water.

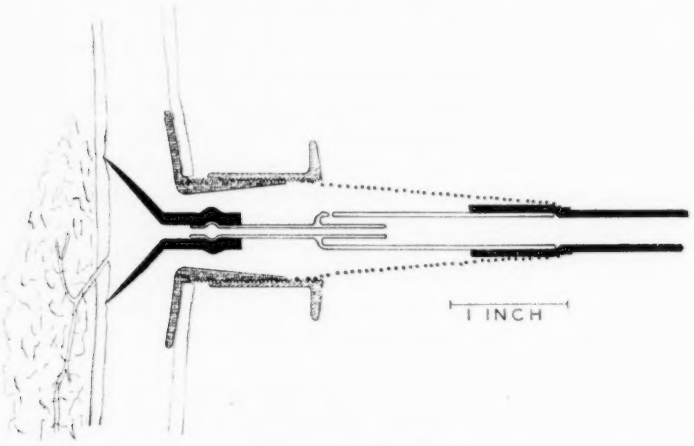

Fig. 1. Sectional view of the apparatus used for collecting pancreatic secretion through a duodenal fistula.

Two arrangements were used for putting test solutions into the intestine which we shall refer to as the injection method and the perfusion method, respectively. In the first a single injection tube was passed through the duodenal cannula to a point about 12 inches below the fistula. Solutions were injected in 20, 40 or 60 cc. amounts. For perfusion, an additional tube, larger and longer than the other, was passed to a point about 22 inches beyond the end of the injection tube. Solutions were delivered into the intestine through the shorter tube by a motor-driven pump at the rate of 15 cc. per minute and, after passing through the upper intestine,

drained out through the longer tube. The solutions were not confined to the regions below the point of injection. In all the experiments in which perfusion was used and in many of the others appreciable amounts of the solutions drained out through the fistula; hence the greater part of the duodenum was exposed to their action.

The solutions used were buffer mixtures of various acids and their sodium salts (the hydrochloride in the case of glycine) in different relative concentrations, depending on the pH. All solutions were 0.15 M except the citrate buffer which was 0.13 M. The stronger acids and the dibasic acids and their salts are approximately isotonic in 0.15 M solutions. The weaker acids are hypotonic in this concentration and to these NaCl was added to bring the Δ to between 0.5° and 0.6°C.

RESULTS. *pH thresholds.* The pH at which the various buffer solutions began to be effective in causing pancreatic secretion was not significantly affected by the method of administering the solution although a slightly higher percentage of positive results was obtained by perfusion than by the injection method. In this respect also the results were the same in the three animals, including the one in which bile was excluded from the duodenum. In table 1 the results are classified as "positive," "negative" or "doubtful." Those classified as "positive" were characterized by an unmistakable increase in the rate of pancreatic secretion which occurred within a reasonable time after the start of the injection or perfusion and for which no other cause than the applied stimulus was apparent. In those classified as "doubtful," an increase in secretion occurred but it was so slight as to be within the range of variation evident during the control period, or it occurred too early or too late to be interpreted as the result of the stimulus, or some other probable cause for the increase was evident. All others are classified as "negative."

The threshold may be defined for our purpose as the pH at which the ratio of positive to negative results becomes greater than 1. These ratios are given in the last column of the table. According to this definition the various thresholds were as follows: HCl, less than pH 3.0; phosphate, between pH 3.0 and pH 4.0; sulphanilic acid, between pH 4.0 and pH 4.5; glycine, between pH 4.0 and pH 4.5; lactate, between pH 4.5 and pH 5.0; acetate, above pH 4.5; glutamic acid, between pH 4.5 and pH 5.0; citrate, above pH 7.0 (?).

From the size of the ratios as given in the table as well as from the magnitude of the responses as shown in the records, it is evident that the threshold for lactate buffers is nearer pH 4.5 than pH 5.0; that for the glutamic acid buffers nearer pH 5.0. Although the citrate buffers were apparently effective in neutral solution, the effect was small and probably negligible. The threshold for citrate acting as an acid is probably not far from pH 5.0.

Differences were also evident in the amount of extra secretion produced by the different buffer solutions at the same pH. For example, consider-

TABLE 1

Summary of results

Explanation in the text

BUFFER	POSITIVE		NEGATIVE		DOUBTFUL		RATIO + TO −
	P*	I†	P	I	P	I	
NaCl + HCl							
N/500 HCl	3	10		5			2.6
N/1000 HCl		1	3	6			0.111
$NaH_2PO_4 + H_3PO_4$							
pH 3.0		7					∞
pH 4.0	1		7				0.142
Sulphanilic acid + NaOH							
pH 3.9–4.1		5				1	∞
pH 4.45–4.65	1	4	2	4	2	1	0.833
pH 5.0		1		3			0.333
Glycine + HCl							
pH 3.9–4.1	8				1		∞
pH 4.45–4.65	3		2	3	1		0.6
Lactic acid + NaOH							
pH 2.3–4.0		7		1			7.0
pH 4.45–4.65	9	35	2	13	1	3	2.93
pH 4.9–5.1		1	5	8			0.076
pH 6.9–7.2		1		5		1	0.2
Acetic acid + NaOH							
pH 4.45–4.65	3	3	1				6.0
pH 6.9–7.0				1			0
Glutamic acid + NaOH							
pH 3.2–4.0		10		1			10.0
pH 4.45–4.65	5	15		3		5	6.66
pH 4.9–5.1	3	4	3	6	1	8	0.77
pH 6.9–7.2				8			0
Citric acid + NaOH							
pH 4.45–4.65	12	7		2		3	9.5
pH 4.9–5.1	4		1	1	1		2.0
pH 6.9–7.2		3		1			3.0

* Perfusion.

† Injection.

ing only the positive results obtained by the injection method at pH 4.5, the lactate buffer produced on the average slightly less than 2 cc. of extra

secretion, the glutamic acid buffer slightly more than 3 cc. and the acetate buffer (3 experiments only) a little more than $3\frac{1}{2}$ cc. Typical results obtained with lactic, glutamic and citric acid buffers at pH 4.5 are shown in figure 2.

In addition to the experiments listed in the table, HCl caused an increase in pancreatic secretion at pH 4.0 when buffered with colloidal

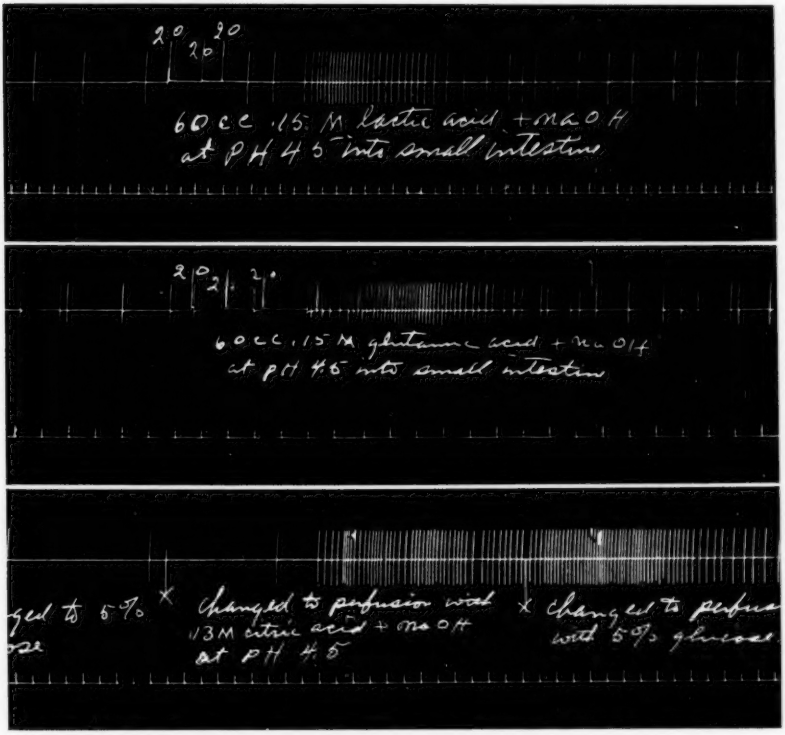

Fig. 2. Typical records showing the effect on pancreatic secretion of various buffer solutions in the intestine at pH 4.5. Upper record, injection of 60 cc. 0.15 M lactate; middle record, injection of 60 cc. 0.15 M glutamic acid buffer; lower record, perfusion for 10 min. with 0.13 M citrate. The drop recorder delivered 17 drops per cubic centimeter. Time is in 30 sec. intervals.

aluminum hydroxide and kaolin. Two proprietary preparations of aluminum hydroxide were used with similar results.

The buffering efficiency of the various solutions. Measurements of the pH of material that drained out of the intestine in perfusion experiments proved not only that the solutions were partially neutralized in the intestine but also that there was a marked difference in the amount of

neutralization of different buffer solutions. These considerations led to an investigation of the buffering capacity of the various solutions within the pH range found to be significant for our purpose. The results are shown in figure 3. A comparison of these titration curves with the data given in the table shows a close correlation between the buffering capacity of the solutions as indicated by the amount of NaOH required to cause a given

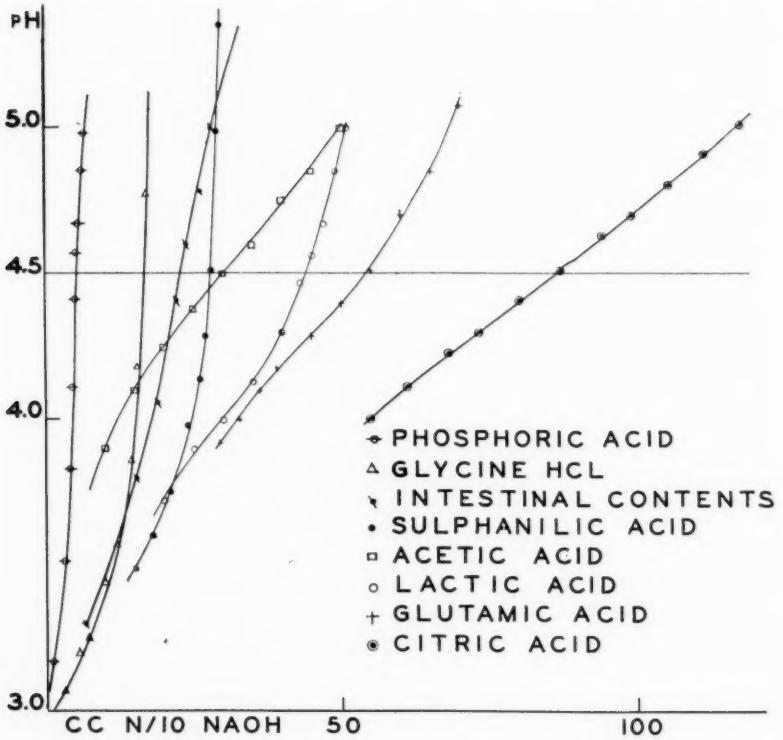

Fig. 3. Significant portions of titration curves of various acids. Fifty cubic centimeters of 0.15 M acid were adjusted to pH 3.0 and titrated to pH 5.0 or above with N/10 NaOH. The lower portions of some of the curves are omitted to avoid confusion. We are indebted to Earl Thomas, Jr. for most of these data.

change in pH and the pH at which they begin to act as stimuli for pancreatic secretion. It appears probable, therefore, that the apparent differences in threshold are caused by differences in the capacity of the solutions to maintain an effective degree of acidity in the intestine rather than to true differences in the threshold for acids of different molecular structure.

This interpretation is borne out by the behavior of glycine and acetic

acid, which are very similar in structure but differ greatly in the pH at which they begin to act as stimuli for the pancreas and differ in a corresponding manner in their efficiency as buffers. Sulphanilic acid, on the other hand, which belongs to an entirely different class of organic compounds is practically equivalent to glycine as a buffer and also as a stimulus for the pancreas within the pH range studied.

The periodic activity of the pancreas. We have made no special study of this phenomenon but find it necessary to refer to it because its occurrence affects the interpretation of our results. As pointed out by Boldyreff (1904) (for other references see Babkin, 1914) the periodic activity of the gastro-intestinal muscle, generally referred to at present as hunger activity, is accompanied by pancreatic secretion. In our dogs this apparently spontaneous secretion occurred in control experiments at intervals of $1\frac{1}{2}$ to 2 hours. Its appearance tended to confuse the results in some instances, particularly when dealing with inert substances. Probably most, if not all, of the "positive" results recorded following the use of substances that were generally ineffective as stimuli are due to "periodic" activity. It is less of a factor in the results obtained with effective stimuli because most of the substances that cause pancreatic secretion also inhibit hunger activity, hence the secretion obtained is not likely to be augmented by "periodic" activity. Since glucose in the intestine tends to inhibit hunger activity and does not stimulate the pancreas, we made extensive use of 5 per cent glucose as a control solution, especially in perfusion experiments.

Discussion. The pH threshold for pancreatic stimulation by means of acid in the intestine appeared to vary between pH 3.0 and pH 5.0 depending on the acid used, but the variations are of doubtful significance. They appear to be fully explained by the demonstrated differences in the ability of the solutions to maintain their acidity in the intestine. It is probably safe to assume that the true threshold is reasonably constant and could be measured accurately if a constant pH could be maintained at the point of contact of the stimulus with the reacting mechanism. A nearer approach to this ideal condition is obtained with the stronger buffers such as citrate or glutamic acid than with the weaker ones such as HCl or phosphate; therefore, the true threshold is probably not below pH 5.0, which was the apparent threshold for the best buffer.

It does not necessarily follow that acidity will be an effective stimulus for the pancreas when the intestinal contents during digestion are no more acid than pH 5.0. The results obtained by the perfusion method show that even when an acid solution in the intestine is continuously renewed at a constant pH the buffering capacity as well as the acidity of the solution is a factor in determining its effectiveness as a stimulus. The buffering of the intestinal contents will, doubtless, vary considerably under different conditions. In one experiment on a dog following a meal of raw meat the

intestinal contents were found to have slightly less buffering capacity than a 0.15 M lactic acid solution (fig. 3). Under these conditions no stimulation due to acid could be expected at pH 5.0 and only a barely perceptible amount at pH 4.5. The practical threshold for bringing about pancreatic secretion at a rate comparable to that normally present during digestion would probably be in the neighborhood of pH 4.0.

Thomas (1940) found that in dogs fed raw meat the contents of the first part of the duodenum varied in acidity between pH 2.4 and 7.0 but were generally near pH 4.0. Therefore, under some physiological conditions the acidity of the intestinal contents during digestion is adequate to stimulate the pancreas.

The fact that the practical threshold for pancreatic stimulation by means of acid and the prevailing acidity of the duodenal contents under the same conditions are alike (pH 4.0) indicates that one function of the acid mechanism is to control intestinal acidity.

SUMMARY AND CONCLUSIONS

1. A method is described for the study of pancreatic function without a pancreatic fistula.

2. An attempt was made to determine the pH at which acid in the intestine begins to act as a stimulus for pancreatic secretion.

3. Variations in pH threshold were observed but these were interpreted as due to differences in the ability of buffer solutions prepared with different acids to maintain their acidity in the intestine.

4. The pH threshold for the more efficient buffers was near pH 5.0. We conclude that the true threshold, if it could be determined, would not be below pH 5.0.

5. The practical acid threshold for causing a significant amount of secretion in the dog digesting raw meat was estimated to be near pH 4.0. This degree of acidity is commonly present in the dog's duodenum during meat digestion.

6. We conclude that under some physiological conditions the acidity of the intestinal contents is adequate to stimulate the pancreas.

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SOME FACTORS AFFECTING THE SPECIFIC DYNAMIC ACTION OF FAT IN NORMAL AND PARTIALLY DEPANCREATIZED RATS

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The elevation of basal metabolism which follows pancreatectomy in dogs and cats has at times been ascribed to the increased activity of the thyroid gland or of the adrenal medulla. Ring and Hampel (1933a) brought evidence to show that a large metabolic increase may occur following the removal of the pancreas in animals lacking the thyroid gland or functional adrenal medulla. Furthermore, the increased protein metabolism of diabetes in our opinion fails to account for the stimulation of metabolism (see Ring and Hampel, 1933b; Ring, 1936). In 1935 Ring showed that in partially depancreatized cats if the basal metabolism is approximately normal, the specific dynamic action of fat is elevated. On the other hand, in more severe diabetes, associated with a high basal metabolism, the calorigenic action of fat disappears. These facts suggest that the processing of fat is greater in diabetes than in the normal state and in fact may be so rapid that the ingestion of fat cannot further increase this processing.

METHOD. For continuing this work we have chosen rats so that the observations could be made on a larger and more homogeneous group. Only animals which would remain quiet for long periods of time were selected. The metabolism was measured by using a modification of Benedict's "Multiple Chamber Respiration Apparatus" which recorded continuously the oxygen consumption of eight rats kept in separate jars. The graphs obtained made it possible to measure the oxygen consumed during periods when the animals were quiet. No determinations of carbon dioxide production were made as previous studies had shown that the respiratory quotients were changed not more than 0.01 or 0.02 as a result of fat ingestion. The estimation of heat production was based on the assumption of a respiratory quotient of 0.72. The surface area formula used was that devised by Lee (1929).

In the first group of experiments, rats were fasted for fifteen hours then placed in the respiration apparatus where the oxygen consumption was recorded continuously for eight hours. Quiet periods each hour were

measured and averaged to obtain the figure for basal metabolism. A week later, the same animals, again postabsorptive, were given 1.5 cc. of oleic acid each by mouth just before beginning the eight hour period of metabolic measurements. These experiments were alternately repeated

TABLE 1

Calorigenic effect of oleic acid in normal rats and depancreatized rats

RAT	BASAL MET. (CAL. PER SQ.M. PER DAY)	INCREASE AFTER OLEIC ACID		BASAL MET.	INCREASE AFTER OLEIC ACID	
		Cal.*	Per cent		Cal.*	Per cent
			Normal			
A	778	59	7.5			
B	787	67	8.5			
C	788	35	4.4			
D	758	20	2.6			
E	789	47	6.0			
F	806	45	5.6			
G	854	52	6.1			
H	807	46	5.7			
I	845	84	10.0			
J	844	60	7.1			
K	844	71	8.4			
Average increase		53.3	6.6			
			Depancreatized			
					After fat diet	
1	721	67	9.3			
2	736	70	9.5			
3	769	48	6.2			
4	746	55	7.4			
5	693	57	8.2			
6	741	60	8.1			
7	665	58	8.0	721	123	17.0
8	762	57	7.5	735	120	16.3
9	736	54	7.3	845	114	13.5
10	636	54	8.5	840	79	9.4
11	727	75	10.3	844	90	10.6
Average increase		59.5	8.2		105.5	13.4

* Increase, given in calories per square meter per day, was determined during a period of eight hours.

during subsequent weeks until there were at least four basal figures and three determinations following the ingestion of oleic acid. It was later found that this procedure could be shortened without reducing the accuracy of results by simply measuring the basal metabolism for the three hours just prior to the ingestion of oleic acid.

In depancreatizing the rats, the operative procedure of Shapiro and Pincus (1936) has been followed. The metabolic studies were begun when these animals had reached a weight of 150 grams or more.

Since numerous observations have shown that the adrenal cortex may play a rôle in the development of typical pancreatic diabetes (see Hartman and Brownell, 1934; Lukens and Dohan, 1938), it is of interest to know how cortical extract affects the specific dynamic action of fat. The Wilson Laboratories kindly supplied us with their preparation. Similar results were obtained using extract prepared in this laboratory. Five cubic

TABLE 2

The specific dynamic action of fat before and after the ingestion of a fat diet for three days

RAT	BEFORE FAT DIET		AFTER FAT DIET	
	Cal.*	Per cent increase	Cal.*	Per cent increase
		Normal		
L	59	7.9	73	9.2
M	67	8.0	69	8.2
N	13	1.7	52	6.3
O	56	7.7	87	11.2
P	68	8.5	58	7.2
Q	57	7.5		
Average.....	53.3	6.9	67.8	8.4
		Depancreatized		
19	56	7.7	93	15.0
12	33	4.7	133	19.4
15	73	9.3	112	14.1
16	45	6.3	85	13.6
17	77	10.8	109	15.5
Average.....	56.8	7.8	106.4	15.5

* Increase given in calories per square meter per day was determined during an eight-hour period after the ingestion of fat.

centimeters of the extract were given intraperitoneally prior to the basal metabolism measurements.

RESULTS. In the left-hand columns of table 1 will be found the averages of results on normal animals using the first procedure (basal metabolism measured for an entire day). Table 2 contains the results using a three-hour preliminary basal measurement. It will be seen that the figures are quite similar using either method. The partially depancreatized rats show almost the same S.D.A. as the normal rats if they are kept on their regular diet (Purina Dog Chow). This, we believe, is because the diabetes produced in the rat by pancreatectomy is very mild. When the operated

animals are placed on an exclusive fat diet (olive oil) for three days in order to increase the severity of the diabetes, then changes are produced which bring about a decided increase in the specific dynamic action of fat during the subsequent weekly measurement even though these rats are eating their regular diet once more (see tables 1 and 2). Normal animals treated in the same way show negligible changes. That the differences found are not due to poor nutritional state of the operated animals, is shown by their

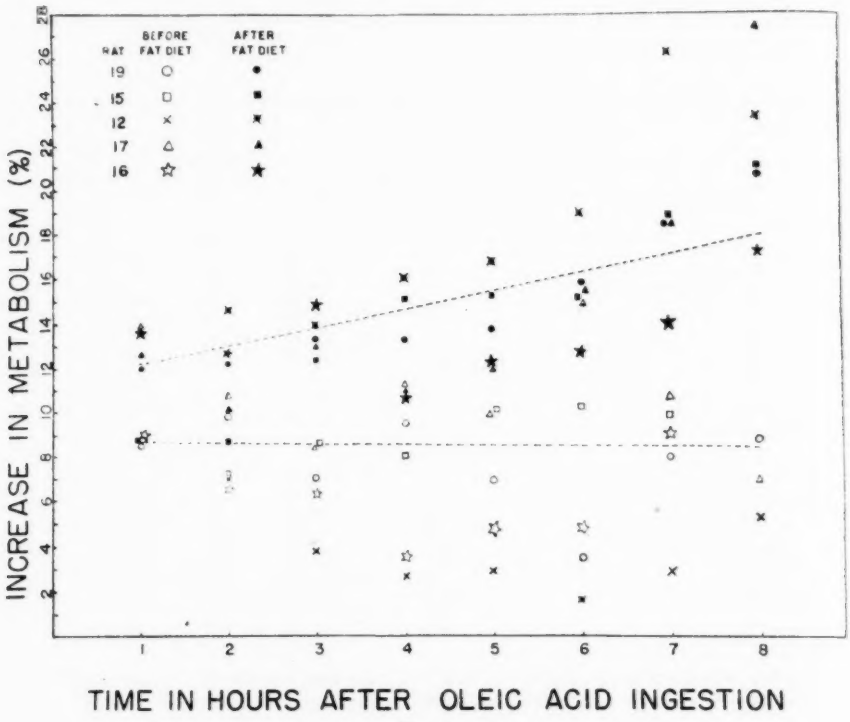

Fig. 1

appearance and also by the determination of the nutritional correction factor of Cowgill and Drabkin $\frac{\text{Weight}^{\frac{1}{3}}}{\text{Body Length}}$. In five normal animals measured, this factor varied between 0.270 and 0.287. Seven operated animals gave figures between 0.271 and 0.302.

Although all animals appeared to be very quiet for a large part of the time they remained in the respiration chamber, it seemed possible that

activity was not properly recorded. In order to check this, three depancreatized and four normal rats were given water instead of oleic acid. The following are the average changes in metabolism of each rat during the next eight hours: +1.3 per cent, +1.2 per cent, +0.4 per cent, +0.3 per cent, −0.5 per cent, −0.7 per cent.

The course of the metabolic changes in the depancreatized rats during the eight-hour period after fat ingestion is plotted in figure 1. The results on normal animals if given here would duplicate those shown for depancreatized rats before the latter were placed on an exclusive fat diet. There is a slight elevation of metabolism which is maintained throughout the period of measurement. The failure of the metabolism to return to the basal level by the end of eight hours is not surprising since about six hours are required to complete the absorption of the fat ingested (see Barnes,

TABLE 3

Effects of cortin on the calorigenic action of fat

NORMAL			DEPANCREATIZED		
Rat	Control*	After cortin†	Rat	Control*	After cortin†
L	61	8	19	94	61
M	73	11	16	78	40
O	48	24	15	96	45
N	67	42	12	110	22
Q	56	00	17	81	59
Average.....	61	19		92	45

* Average of four measurements of calorigenic action of fat in weeks preceding and following cortin injection, given in calories per square meter per day.

† Average of at least two determinations.

Wick, Miller and MacKay, 1939) and the calorigenic effect must last for some time after this. A few of our experiments suggest that as large a calorigenic effect is obtained with a smaller dose of oleic acid (1 cc.). After the depancreatized rats have been kept on the fat diet for three days, the S.D.A. during subsequent weekly measurements is larger and the metabolism may continue to rise throughout the eight hour period. Three animals studied eighteen hours after fat ingestion still showed an increase above basal metabolism of 12 per cent, 15 per cent and 16 per cent.

The effect of cortical extract on the calorigenic effect of fat is shown in table 3. It will be seen that the S.D.A. is depressed in both normal and depancreatized rats. It is believed that the S.D.A. of fat is due to the processing of this material in the liver. This processing is, no doubt, less when there are sizable glycogen stores. Many have shown that liver glycogen is increased after the injection of cortical extracts (see Russell,

1940, and this may well explain the results which were obtained. The diabetic animal with less store of carbohydrate, processes more fat and the S.D.A. is not depressed to as low a level by extract.

CONCLUSIONS

1. In rats, the specific dynamic action of fat is not affected by pancreatectomy unless the animals are on a fat diet for at least three days. Then the calorigenic action of fat in the operated animals is approximately doubled. The fat diet does not greatly affect the S.D.A. in normal animals (see tables 1 and 2).

2. Since the partially depancreatized rats studied were not undernourished, nutritional condition cannot be responsible for the results obtained.

3. Giving large doses of cortical extract depresses both the S.D.A. of fat of normal and depancreatized rats (see table 3).

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THE RELATIONSHIP BETWEEN SALT INTAKE AND THE POLYURIA OF EXPERIMENTAL DIABETES INSIPIDUS

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Functional deficiency of the posterior lobe of the pituitary is characterized not only by an augmentation of the water exchange, but also by a disturbance in the salt metabolism. Evidence that diabetes insipidus is accompanied by some derangement of the salt regulating mechanism includes the following observations: there is a delay in the excretion of salt when it is ingested in large amounts by diabetes insipidus patients (1), there is a relative inability of the kidney to concentrate salt (2, 3), and animals with diabetes insipidus do not show the changes in serum sodium and chloride following adrenalectomy which are characteristic of non-polyuric animals (4).

More direct evidence of a definite relationship between salt metabolism and diabetes insipidus is seen in the work of Swann (5, 6, 7). Working with hypophysectomized rats, Swann concluded that the polydipsia of diabetes insipidus is secondary to the disturbance in salt regulation. He ascribed the loss of polydipsia which occurs during fasting to the absence of salt intake in the fasted animal, for he found that in hypophysectomized rats the polydipsia was markedly reduced on a diet nearly salt-free, but persistent if salt was given to a fasting animal. This work leads to the view that with low salt intake there would be no polyuria or polydipsia; with moderate salt intake, a moderate polyuria; with high salt intake, a high degree of polyuria. If these findings on the hypophysectomized rat can be shown to be of general application, then they are of very great importance, because diabetes insipidus then becomes mainly a disturbance in sodium chloride metabolism, and in order to control the polyuria and polydipsia it would be necessary only to limit the salt intake of an individual suffering from this disease. We have, therefore, sought to obtain information concerning the relationship which may exist between salt intake and the polyuria of diabetes insipidus, using as test animal the cat with interrupted supraoptico-hypophyseal tracts. The general methods for production of the experimental diabetes insipidus (d.i.) and for care of the animals have been mentioned elsewhere (4).

OBSERVATIONS. Table 1 shows that a close parallelism can be demon-

strated between salt intake and urine output in diabetes insipidus, when the NaCl intake is on a high level. The cat shown in the table, a typical example of several experiments, received 1.5 grams of sodium chloride daily added to his food, and in addition he was given 0.5 per cent NaCl solution to drink. This solution was kept continuously in front of the animal instead of water, and the intake was voluntary. Normal animals could not be induced to take in such large quantities of salt.

TABLE 1

Effect on urine volume of adding 1.5 grams of NaCl to meat and substituting 0.5 per cent NaCl solution for drinking water, in a cat with diabetes insipidus

NORMAL DIET			HIGH SALT REGIMEN		
Date	NaCl intake	Urine volume	Date	NaCl intake	Urine volume
	grams	*cc.*		*grams*	*cc.*
1/21	0.238	460	1/25	2.738	575
22	0.238	470	26	4.988	750
23	0.238	525	27	3.238	640
24	0.238	415	28	6.488	1225
			29	12.238	1920
			30	9.988	1660
			31	12.738	2160

TABLE 2

Effect on the urine volume of adding one to two grams of salt to the daily diet

KIND OF ANIMALS	NUMBER OF ANIMALS	DAILY AVERAGES						
		Normal diet			High salt diet			
		Urine volume	NaCl intake	Days	Urine volume	NaCl intake	Days	Increase in urine volume on high salt
		cc.	*grams*		*cc.*	*grams*		*cc.*
Normal	2	119	0.321	12–14	137	1.238	6–7	18
	5	127	0.271	12–15	170	2.158	6–36	43
Diabetes insipidus	3	569	0.238	5–13	760	1.240	10–41	191
	2	705	0.238	10–13	973	2.120	14–17	268

Table 2 shows the difference between the responses of normal and of d.i. cats to the addition of a moderate amount of sodium chloride in the diet, while the animals are allowed water ad libitum. One to two grams of extra salt daily makes very little difference in the urine volume of normal cats, but greatly exaggerates a polyuria already present in the d.i. animals. This confirms some of Swann's observations, as well as the results of similar experiments by Fisher, Ingram and Ranson (8). The effect

of ingested salt in increasing the polyuria of diabetes insipidus is undoubtedly related to the relative difficulty with which d.i. animals concentrate salt in the urine.

Although it is apparent from the results of the salt-feeding experiments that there is a positive relationship between salt intake and the degree of the polyuria when the salt intake is high, it does not follow that the polyuria ordinarily observed in diabetes insipidus is likewise related to the salt which is contained in the ordinary diet.

Table 3 gives a protocol of a cat which was placed on a diet almost entirely free of chloride. This was our regular cat ration of ground beef, with the salt extracted by boiling distilled water according to the method described by Swingle et al. (9). This "salt-free" diet seems to be rather unpalatable, and after a variable number of days the cats refuse it, and go

TABLE 3

Effect of "salt-free" diet and of fasting on the polyuria of a cat with diabetes insipidus

DATE	NaCl INTAKE	URINE VOLUME	URINE SP. GR.	REMARKS	DATE	NaCl INTAKE	URINE VOLUME	URINE SP. GR.	REMARKS
	grams	*cc.*				*grams*	*cc.*		
8/10	0.238	340	1.006	Regular	8/19	0.243	355	1.008	"Salt-free" diet,
11	0.238	340	1.010	diet	20	0.183	460	1.010	with NaCl
12	0.238	365	1.009						added
13	0.238	380	1.006		21	0	95	1.009	Fasted, water ad
14	0.238	260	1.005		22	0	70	1.015	lib
					23	0	135	1.012	
15	0.005	460	1.009	"Salt-	24	0.212	210	1.004	Regular diet
16	0.005	370	1.007	free"	25	0.238	300	1.010	
17	0.005	435	1.008	diet	26	0.238	400	1.009	
18	0.005	355	1.011		27	0.238	450	1.005	

into a voluntary fast. It will be noted from the table that although the diet is almost completely free of chloride, the animals maintain their polyuria so long as they eat it, and the addition of salt in the amount present in the normal daily food intake does not affect the polyuria. On the very first day of fast, however, the urine volume drops to a level nearly corresponding to that which is characteristic of the normal animal. Since the polyuria was maintained on a diet practically free of salt, it was evidently not the lack of salt which caused the reduction in the urine volume when the fast started. The figures for the urine specific gravity were throughout the period at a level characteristic of the d.i. cat, even on the days of fast; so, judged by this criterion, the animal still had diabetes insipidus even on the days when there was no obvious polyuria. Similar experiments were performed on four cats altogether, but as the other re-

sults were fully confirmatory of those shown, they are omitted from the table.

Another experiment on a fasting d.i. cat is summarized in figure 1. In the first fasting period shown, the fast was complete except for water, which was freely available. It may be noted that during this period there was a prompt reduction in urine volume. The second fasting period was the same as the first, except that the salt intake was kept constant by the addition of a small amount of salt to the drinking water. Yet, in spite of the constant salt intake, the drop in urine volume was as marked and as prompt as before. Comparable experiments performed on two other animals gave fully concordant results.

On the basis of the experiments summarized above, we conclude that the d.i. cat does not react to these experimental conditions in the same way as has been reported for the hypophysectomized rat. The decrease in

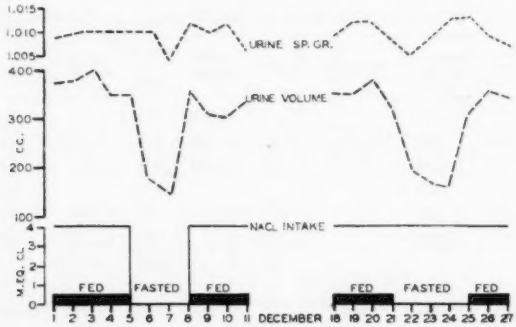

Fig. 1. For explanation see text

urine volume observed during the fasting of d.i. cats is not due to the salt deprivation which accompanies fasting, nor is the polyuria of diabetes insipidus solely dependent on the salt content of the normal diet. If there is any element in the diet which is responsible for the polyuria, it is not the salt alone.

SUMMARY

Cats with diabetes insipidus produced by interruption of the supra-optico-hypophyseal tracts show a close parallelism between salt intake and urine volume when the NaCl intake is on an abnormally high level, but this parallelism does not hold for salt intakes of normal or below normal levels.

During fasting, the urine volume of a diabetes insipidus cat falls almost to the level which is characteristic of a normal animal. The loss of the polyuria is not due, however, to the salt deprivation which accompanies fasting, because if the salt intake is kept at a normal level during the

fast by adding salt to the drinking water, the urine volume falls just the same. Furthermore, the animal maintains his polyuria practically intact when placed on a diet almost completely free of salt.

It is evident, therefore, that if there is any factor in the ordinary diet which causes the maintenance of the large fluid exchange of diabetes insipidus, it is not the sodium chloride alone which is responsible. We conclude that the claim which has been made for the hypophysectomized rat, that the polyuria is dependent on the salt in the ordinary diet, can not be valid for the d.i. cat.

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THE EFFECT OF DISTENTION ON BLOOD FLOW THROUGH THE INTESTINE

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It would be expected that any increase in extra-capillary pressure would increase the resistance to blood flow by narrowing the smallest vessels. The behavior of the pulmonary bed conforms to this expectation (De Jager, 1885). The apparent lack of conformity of the intracranial bed has not yet been adequately explained (Ferris, 1939). There is no question that a rise in intra-enteric pressure from whatever cause elevates extra-capillary pressure in the walls of the intestine. A passive rise in intra-enteric pressure might be expected to interfere with blood flow somewhat less than a rise due to muscular activity, since during distention the submucosa and inner muscle layers probably to some extent protect the more peripheral layers (Mall, 1896). This may, however, be offset by angulation of vessels in the over-distended intestine (Eisburg, 1925). It is doubtful, therefore, if data relating blood flow to the pressure developed within the intestine by muscular activity (Anrep, Cerqua and Samaan, 1934) can be used in predicting the effect on flow of pressures developed within the intestine by inflation.

Van Zwalenburg (1907) observed stoppage of flow in a few capillaries in the dog's intestine when intra-enteric pressure was raised by inflation to 30 mm. Hg. No data were given on the effect of lower pressures. Gatch and Culbertson (1925), Gatch, Trusler and Ayers (1927), and Dragstedt, Lang and Millet (1929) reported a reduction in venous outflow from the distended intestine of the dog proportional to the elevation of lumen pressure. In preliminary experiments (Lawson and Chumley, 1940) we failed to obtain complete confirmation of these observations, since we found no sustained reduction in blood flow with pressures below a critical level which was usually about 30 mm. Hg. The present report extends these observations, and demonstrates the existence of intrinsic compensating mechanisms through whose operation the flow of blood through the intestine may be capable of being maintained without reduction in the face of elevated lumen pressure.

METHODS. Loops of ileum 6 to 12 cm. long were isolated between ligatures in barbitalized dogs. Arterial flow into the loop was measured

by differential manometry, using a modification of the technique described by Lawson and Holt (1939). The superior mesenteric artery was dissected out for 1 to 2 cm. above and below the origin of the branch supplying the loop, and the constricting clamp was placed on the artery central to the branch. A second branch, below the constriction, was cannulated and connected with one limb of the differential manometer, and all other branches below the constriction were ligatured. The carotid, the femoral, or a convenient branch of the superior mesenteric artery above the constriction was cannulated and connected with the other limb of the manometer. A mercury manometer was connected through a T in this line to give an absolute reading of systemic arterial pressure.

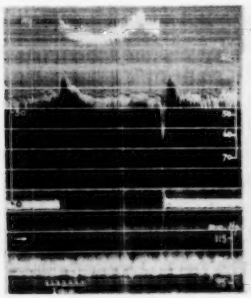
A differential metal bellows manometer was used for recording the fall in pressure across the constriction in most of the experiments (Lawson, 1940). This system, to give satisfactory records, requires a lowering of pressure in the artery below the constriction of 10 mm. Hg or more. Since the level of pressure in the intestinal arteries is undoubtedly a factor in determining the effect of distention on blood flow, an inverted air-water manometer of the type described by Wagoner and Livingston (1928) was used in a portion of the work for comparison. The central limb of the manometer was expanded to form a well, and optical records were made of the changes in fluid level in the peripheral limb. With this type of manometer satisfactory records were obtained with mean pressure in the intestinal arteries (below the constriction) only 0.5 to 3.5 mm. Hg below mean systemic arterial pressure. Results with the two types of manometer were qualitatively similar. No quantitative comparison was attempted.

Calibration of the flow-meter to obtain data in terms of volume flow was not done routinely. Since slight changes in the tonus of the artery or deposition of fibrin at the constriction may change the calibration of the flow-meter, the calibration to be valid should be repeated at intervals during the experiment. As this requires shutting off the artery supplying the loop and opening a second artery below the constriction for collecting blood, it entails some damage to the intestine, and was usually not done. The records show therefore only the pressure difference across the constriction. Assuming that the dimensions of the constriction and the viscosity of blood remain constant over the short periods occupied by each record, an increase in the pressure difference means an increase in blood flow, and *vice versa* (Lawson and Holt, 1939). Without proof that the calibration of the flow-meter has remained constant, the records cannot be used for comparing the amount of flow in widely separated portions of the same experiment.

In the preliminary studies the loop of intestine was distended by inflating with air a large condom balloon lying within the lumen against a water or mercury manometer. In the later experiments pressure at any

level of distention was kept constant by inflating either the balloon or the loop directly with water from a leveling bottle. No inflations above 60 mm. Hg were studied. It is doubtful that pressure within the dog's small intestine rises above 40 mm. Hg even in experimental intestinal obstruction (Antoncic and Lawson, 1940). The effect of distention prolonged beyond 5 minutes was not studied.

RESULTS. Typical changes in blood flow during and following a period of inflation are shown in figures 1 and 2. For descriptive purposes five phases in the response may be recognized.



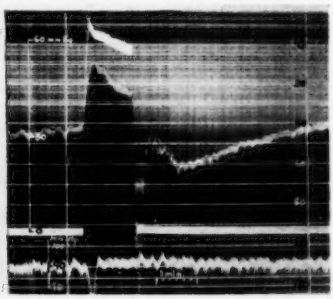


Fig. 1 (left). Records from above down are: 1, pressure difference in mm. H_2O between carotid and mesenteric arteries across constricting clamp, recorded optically from air-water manometer (see text). An ascending record indicates a fall, and a descending record a rise in the pressure difference. 2, pressure within loop of ileum, inflated during the period of sudden elevation against a water manometer. 3, carotid arterial pressure recorded from a mercury manometer. The response to inflation is typical except for the absence of phase 5 (see text).

Fig. 2 (right). Construction of figure as in figure 1. The record shows exaggerated post-inflation hyperemia (phases 3-5, see text) following brief, moderately high pressure inflation.

Phase 1. Flow was reduced at the moment of inflation, usually reaching its greatest reduction within 8 to 10 seconds after the inflation was complete.

Phase 2. There was always a tendency for flow to recover its control value during sustained inflation, even though lumen pressure was kept constant by distending the intestine from a leveling bottle rather than against a manometer (fig. 4). The rate of recovery during this phase was usually rapid during the first 10 to 15 seconds, then slower until a plateau was reached within 1 to 3 minutes of the inflation. Usually with distending pressures below 30 mm. Hg (fig. 6) and sometimes with pressures up to 60 mm. Hg (figs. 1, 4) the recovery of flow during this phase was complete, flow during the remainder of the distention period continuing at control levels.

Phase 3. On deflation there was typically a sudden increase in flow over control levels, the peak of the increase being reached at the time of complete deflation. With brief or low pressure inflations this phase usually terminated the response, flow gradually declining to its control value within 5 to 90 seconds (fig. 6, 1st inflation). The hyperemia of phase 3 was observed even though flow had fully recovered its control value during phase 2 (fig. 6, 1st distention) and in animals in which distention caused an increase in flow (fig. 5).

Phases 4 and 5. With longer or larger distentions the period of post-inflation hyperemia was prolonged, the greatest duration observed in our studies being 6 minutes. Under these conditions the hyperemia was usually interrupted within 15 to 30 seconds of its beginning by a period of reduced flow lasting 10 to 50 seconds (phase 4) during which flow was

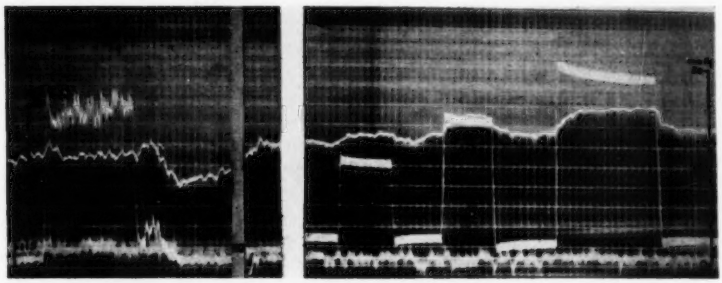


Fig. 3. Construction of figure as in figure 1. Time in 10-second intervals. A (left), effect of inflation on the freely distended loop, before application of plaster cast. Air trapped in balloon on deflation records hypermotility during phase 4 of the response (see text). B (right), effect of three inflations of varying degree after application of plaster cast.

sometimes less than during the control period (fig. 2). Records of intestinal muscular activity made by incompletely deflating the gut showed intense motor activity at this time (fig. 3 A). Rhythmic variations in flow, synchronous with the beat of the intestine, with flow at its minimum value at the peak of each gut contraction, were often observed. The fifth phase consists of a resumption of hyperemic flow levels following the termination of phase 4, with gradual return to the control (fig. 2).

The response to pressure without distention. It would be expected that an initially reduced blood flow would decline still further as the intestine distends, and as pressure in the outer layers of the gut wall rises. Since, instead, flow always increased during phase 2 as the intestine enlarged, it was decided to prevent the enlargement of the loop by encasing it in plaster-of-Paris.

A comparison of the response of the same loop before and after ap-

plication of the cast showed striking differences (fig. 3). In every case the jacketed loop showed a simple monophasic reduction in flow, persisting throughout the period of inflation, with no tendency to return toward the control. On deflation from moderate inflation levels (below 40 mm. Hg) the control flow was immediately resumed, with no suggestion of any part of the post-inflation period of hyperemia (phases 3–5) typical of the dis-

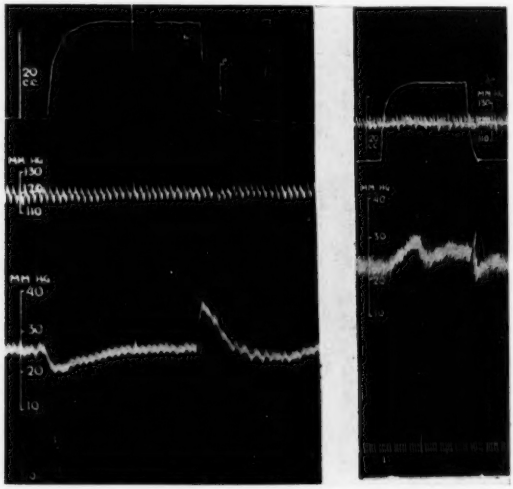


Fig. 4 (left). Records from above down are: 1, volume of water accepted by loop of ileum distended from leveling bottle at height of 54 cm. H_2O (40 mm. Hg). Record made with volume recorder connected above water level in leveling bottle; 2, carotid arterial pressure; 3, pressure difference in millimeters of mercury between carotid and mesenteric arteries across constricting clamp, recorded with differential metal bellows manometer. An ascending record indicates a rise, and a descending record a fall in the pressure difference. Time in intervals of 10 seconds and 1 minute. A reference ordinate has been erected at the point where the pressure difference levels off at its control value, approximately 20 seconds after full enlargement of the loop.

Fig. 5 (right). Construction of figure as in figure 4, except that the two uppermost records are in inverse order. To show increased blood flow through loop distended under pressure of 23 mm. Hg. Note well-marked phase 3, and slight phase 5 on deflation (see text).

tended loop. That the jacketed loops were capable of reactive hyperemia was proven by clamping the artery for 90 seconds or more. Hyperemia was also elicited following inflations above 80 mm. Hg lasting 2 to 3 minutes.

The response to stretch. Since a comparison of the freely enlarging with the encased loop demonstrates that enlargement of the intestine is in some way responsible for the maintained blood flow during inflation, it should

be possible to stretch the intestine in such a way as occasionally to produce a pure increase in flow. In the hope that the compensating mechanism might be set in operation without excessive increase in extravascular pressure in the gut wall, loops were laid open along their antimesenteric border, the cut edges placed in bulldog paper clips, and stretched transversely by hand. In one animal flow was increased during stretch on every trial. In three animals an initial increase in flow progressively deteriorated, and at the end of approximately one hour flow was consistently reduced by

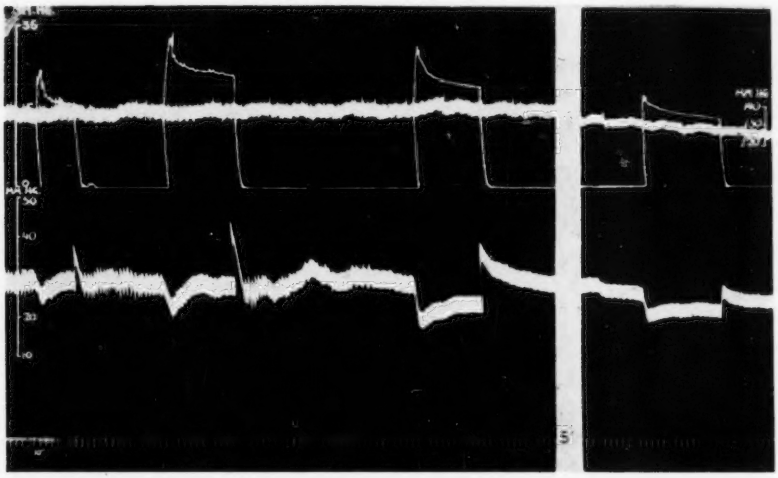


Fig. 6. Construction of figure as in figure 4, except that second record from top is a manometer tracing of inflation pressure. There is complete restoration of blood flow during the first inflation (initial pressure approximately 25 mm. Hg) and nearly complete restoration during the second (initial pressure approximately 35 mm. Hg). During the third inflation to approximately the same height as the second, but following injection of 1 per cent cocaine hydrochloride into the lumen of the loop at signal, there is less complete restoration of flow. There is almost no restoration of flow, and almost no post-inflation hyperemia following the fourth inflation, smaller than either of the first two.

stretch. In the remaining animal, a decrease in flow was obtained initially. After local application of 0.1 per cent pilocarpine, stretch caused an increase in flow, which was abolished after local application of 0.1 per cent atropine (fig. 7).

The effect of decentralization on the compensating mechanism. Perivascular section of the mesenteric nerves was without immediate effect on any phase of the response to distention. In two animals, however, whose loops had been decentralized an hour or more before starting the experiment

and in which the intestine was active and in high tonus, all distentions below 50 mm. Hg produced an increase in flow over the control during the inflation period (fig. 5). A similar increase was obtained in two other animals in our series, without denervation. In all these, the post-inflation behavior (phases 3–5) resembled that of loops whose flow was reduced during distention.

The effect of local anesthetics on the compensating mechanism. Compensation during phase 2 was either wholly abolished, or greatly reduced by application of 1 per cent cocaine hydrochloride to the mucosa of the loop (fig. 6). In every case the maximum distention pressure for which full

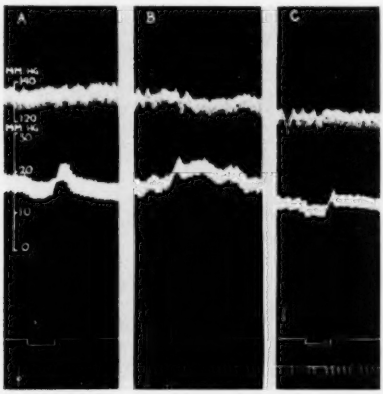


Fig. 7. Records from above down are: 1, carotid arterial pressure; 2, pressure difference between carotid and mesenteric arteries as in figure 4. The signals mark the application of transverse stretch to the loop of ileum (see text for method). In each trial the loop was stretched to a measured width of 4 cm., regardless of tonus or control width. A, initial response of untreated loop. B, after application of 0.1 per cent pilocarpine nitrate to the mucosa of the loop. C, after application of 0.1 per cent atropine sulfate to the mucosa.

compensation could be effected was markedly lowered after cocaine. Post-inflation hyperemia following moderate distentions was either greatly reduced or entirely lacking in cocainized loops, although these loops responded to temporary arterial occlusion with hyperemia on restoration of flow. The response of the fully cocainized loop thus closely resembled the response of the loop encased in plaster-of-Paris.

There was also a reduction of the maximum pressure permitting full compensation in loops treated with 1 per cent procaine hydrochloride, although complete abolition of compensation during phase 2 was never attained. There was not as complete suppression of the post-inflation hyperemia as after cocaine.

DISCUSSION. The over-reduction of flow at the moment of inflation (phase 1) and the over-increase of flow at the moment of deflation (phase 3) were tentatively interpreted in the earlier communication as the result of pressure changes in the capillary area due to capacity alterations on compression and decompression (Lawson and Chumley, 1940). This interpretation was supported by the demonstration of simultaneous changes in the opposite direction in venous outflow at these times. The absence of these phases in the response of the intestine encased in plaster or treated with cocaine necessitates a re-interpretation, since these procedures would not abolish such capacity changes. That during inflation blood is expressed from the distending gut at the same time that arterial inflow is most sharply reduced, and that during deflation blood pools in the gut during the time that arterial inflow is greatest, is clear from the earlier data. But these demonstrated changes in the volume of blood pooled in the intestine and the attendant changes in the pressure gradient do not appear to affect the resistance to arterial flow. It is possible that they occur for the most part in the veins.

These phases in the response assume new significance in the light of the present demonstration that a compensating mechanism is set in operation by distention of the intestine. Phase 1 probably represents the mechanical reduction in flow due to the increased extravascular pressure, since the flow level reached during this phase remains constant if the compensating mechanism is silenced by encasing the gut or treating it with cocaine. Phase 3 probably represents, in part at least, the persistent action of the compensating mechanism after extravascular pressure has returned to normal, since it is not seen unless some compensation has been elicited. This interpretation of phase 3 is supported by the observation that the increased flow set up by transverse stretch of the intestine only slowly declines after cessation of stretch (see fig. 7B).

In the earlier report a portion at least of the post-inflation hyperemia was tentatively identified as reactive hyperemia. Both the cocainized intestine and the intestine encased in plaster are capable of reactive hyperemia following complete arterial occlusion or excessive inflation. They do not, however, appear to repay the flow deficit incurred during moderate inflations, even though this is greater than the deficit incurred in the untreated, freely distended gut. The part played by reactive hyperemia in the post-inflation behavior of the freely distended untreated intestine is therefore not clear, and will require a quantitative study of reactive hyperemia in the intestine for elucidation.

The site of the compensation cannot be determined from these data. Dragstedt, Lang and Millet (1929) observed a residual flow of blood from the mesenteric veins which no amount of intra-enteric pressure could stop. This was interpreted as passing through mesenteric anastomotic channels.

They reported that this flow gradually increased during prolonged distention until it nearly equaled the original, presumably due to dilatation in the mesenteric channels. Their observations appear to have been made with intra-enteric pressure well above mean arterial pressure, to ensure that no blood could flow through the gut walls. We have not repeated this work. It does not appear likely, however, that we have elicited this phenomenon, since in our experiments flow reaches a steady state short of full compensation with distentions above a certain critical level, but well below the level of mean arterial pressure. Our data show only that total flow through the system intestine-mesentery returns to normal during distentions below the critical level, and offer no evidence against drastic redistributions of flow within this system.

The data offer more valid evidence regarding the nature of the compensating mechanism. It is clear that stretch of the intestinal walls either mechanically reduces the resistance to flow, or sets in operation a resistance-lowering mechanism. Acceptance of the first alternative would have to be based on the assumption that in the undistended intestine resistance to blood flow is kept high by angulation and tortuosity of vessels. Were this the case, it would be expected that restoration of flow during the phase of compensation would closely parallel the enlargement of the intestine. Our records fail to support this interpretation, since during phase 2 flow often continues to increase after the intestine has reached its maximum enlargement (see fig. 4), and since full compensation may be maintained throughout phase 2 in the face of a delayed tone rise in the intestine which reduces its volume (see fig. 1).

The second alternative seems the more probable. The mechanism set in operation by stretch might conceivably be entirely extravascular, if the following conditions, most of which are improbable, existed: *1*, the total blood flow through the system intestine-mesentery were reciprocally related to the tonus of the outer layers of muscle in the intestinal wall; *2*, the tonus of these outer layers were inhibited by stretch; *3*, the inner layers of the intestinal wall resisted stretch to the extent that during distention (or stretching) extravascular pressure in the outer layers, under the operation of mechanism *2*, actually fell below normal. A vascular mechanism fits the data equally well, and involves less unlikely assumptions. It leaves unexplained only the apparent relationship of the compensating mechanism to intestinal muscle tone, which is being studied further.

It is suggested as a working hypothesis that the stretching of the intestinal walls during distention sets in operation, through the peripheral nervous apparatus, a mechanism which compensates for the rise in extravascular pressure. The ultimate compensating mechanism is probably dilatation of arterioles. Assuming that no redistribution of flow occurs in the distended intestine, such a mechanism would permit a rise in peripheral intravascular pressure so as to prevent compression of the smallest vessels.

On the basis of this theory, the critical level of distention for which compensation could be effected would be determined by the maximum elevation of capillary pressure permitted by full arteriolar dilatation, as well as by the irritability and efficiency of the compensating mechanism itself.

SUMMARY AND CONCLUSIONS

Blood flow through loops of the small intestine of dogs anesthetized with barbital is only momentarily interrupted by inflation of the loop under pressures below 30 mm. Hg. Higher inflation pressures below mean mesenteric arterial pressure cause an initial marked reduction in flow, from which there is partial recovery within a few seconds.

Simple transverse stretching of a strip of intestine opened along its antimesenteric border sometimes causes a simple increase in flow through the strip.

If enlargement of the loop is prevented by encasing the loop in plaster-of-Paris, or if the loop is treated with cocaine or procaine, any rise in lumen pressure causes reduction in flow which persists without any tendency toward recovery, or with reduced recovery, throughout the inflation.

On deflation of the untreated loop a period of hyperemia ensues, sometimes interrupted early by a period of reduced flow apparently due to deflation hypermotility. The increased flow following deflation appears not to be identical with reactive hyperemia in its mechanism, since it is abolished by treating the gut with cocaine or encasing it in plaster, procedures which augment the flow deficit incurred during inflation, and which do not prevent reactive hyperemia following arterial occlusion.

No phase of the response to inflation is significantly modified by perivascular mesenteric denervation.

It is suggested as a working hypothesis that stretching the walls of the intestine during inflation sets up vasodilatation through intrinsic nervous mechanisms, resulting in local circulatory compensation for the added resistance to flow.

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EFFECTS OF INJECTION OF EXTRACT OF YEAST ON GASTRIC SECRETION IN DOGS[1,2]

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We have prepared a concentrated aqueous acid extract of yeast which, when injected intramuscularly, increases the volume and acidity of secretion by previously resting stomachs of dogs. A similar preparation from spinach has been reported by Bickel (1917), and Gleichmann (1934) observed gastric stimulation in dogs when filtered tomato juice was injected intramuscularly. In no case has the gastrin-like principle been isolated, although the active material from spinach has been characterized as stable to dry heat up to about 140° and to boiling with concentrated hydrochloric acid.

METHODS AND GENERAL PROCEDURE. *Preparation of yeast extract.* Commercial foil-wrapped cake yeast was extracted exactly according to the procedure of Keeton and Koch (1915) for extraction of "gastrin" from animal tissues. The final dry residue was taken up in water so that 1 cc. of solution represented 10 grams of original yeast.

Animals.[3] Our data represent 6 dogs, weighing from 8 to 13 kilos. Three dogs were prepared with a gastric fistula of the Spivack type, while in 3 dogs the gastric fistula was made with an isolated section of jejunum. These 6 dogs gave negative tests for free hydrochloric acid and very low pepsin values in more than 50 per cent of control gastric samples. In 4 additional dogs consistently positive effects upon gastric secretion were obtained by injection of histamine or the yeast extract. However, since the latter dogs regularly secreted an acid gastric juice in control periods, data of these experiments are not included.

Experimental. In all experiments the dogs had received no food for 16 hours. Gastric contents were collected by a syringe and a no. 20 rubber catheter inserted through the fistula. At the beginning of an experiment, and at 15 minute intervals during the succeeding 2 hours, the

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[3] The authors wish to thank Dr. R. A. Bussabarger, Dr. M. J. Tendler and Dr. I. F. Tullis, Jr., for their aid in performing the operations.

stomach was thus emptied. The 2 samples collected during each 30 minute interval were combined for analysis. At the end of the first 30 minute interval, which served as control, the injection of test material was given intramuscularly.

Analytical methods. For *free hydrochloric acid*, 2.5 to 5.0 cc. of the gastric sample were titrated to pH 2.4 (thymol blue indicator); in the same sample *total acid* was measured by further titration to the second color change of thymol blue at pH 8 (Harrison, 1930). *Pepsin* was estimated in the gastric samples when sufficient additional material was available. The method for pepsin was adapted from the amino titration procedure of Linderstrøm-Lang (1928) and the method of Andersen (1938). One cubic centimeter of the gastric sample was incubated at 38° for 100 minutes with 5.0 cc. of 5 per cent sodium caseinate and 1.00 cc. of 0.75 N hydrochloric acid. A control incubation was made with 1.00 cc. of the gastric sample which had been heated in a boiling water bath for 2 minutes. Peptic activity was related to the difference in amino titration values of the two systems. One pepsin unit has been defined arbitrarily as that amount which effects the liberation of one-tenth milliequivalent of amino acid under the specified condition.

RESULTS. Averages of data from 12 experiments in which 3 or 4 cc. of yeast extract[4] were injected intramuscularly are shown in table 1. In the same table are included for comparison the average data of 16 control experiments on the same dogs in which sterile 0.9 per cent sodium chloride solution instead of yeast extract was injected, and the average results of 16 experiments in which 0.1 mgm. of histamine was given intramuscularly. Probable errors of the means are recorded in table 1 for all the volume, free hydrochloric acid, and total acid data and for the pepsin data of the first 30 minute period after injection. The remaining pepsin data are from so few observations as to be useless for statistical analysis.

As compared with gastric response to control injections of 0.9 per cent sodium chloride solution, significant increases occur in volume, free hydrochloric acid, and total acid of the samples collected during the first 30 minute period after injection of yeast extract or histamine. Any differences between the effects of yeast extract and of histamine during this period are of doubtful significance on the basis of this series of data. Free hydrochloric acid and total acid secretion during the second 30 minute period after histamine is still significantly above control levels, but increase in average volume for this period is probable only in the yeast extract experiments. All other apparent variations from the controls in acidity or volume, in any period, are not statistically significant.

[4] Orientation experiments had shown that smaller doses of yeast extract frequently failed to produce a secretory response.

In our judgment, pepsin changes are of doubtful meaning in all experiments, although average pepsin values during the first 30 minute period after injection of yeast extract represent a probable increase as compared with controls. Such dogs are capable of secreting 10 to 20 units of pepsin within each of three consecutive 30 minute periods following a more specific stimulus which does not evoke a correspondingly larger secretion of acid. The values for pepsin noted in table 1, small as they are, may be attributed in part at least to mechanical stimulation by the catheter,

TABLE 1

Effects of injection of 0.9 per cent sodium chloride, histamine, and yeast extract on gastric secretion

INJECTION	VOLUME	FREE HCl	TOTAL ACID	PEPSIN
30 minute period preceding injection				
	cc.	*meq.*	*meq.*	*units*
0.9 per cent NaCl	7.3 ± 1.4	0.01 ± 0.00	0.27 ± 0.04	0
0.1 mgm. histamine	10.1 ± 1.9	0.03 ± 0.01	0.47 ± 0.10	0
3 or 4 cc. yeast extract	5.4 ± 0.8	0.06 ± 0.03	0.61 ± 0.23	0.9
First 30 minute period after injection				
0.9 per cent NaCl	7.1 ± 1.6	0.02 ± 0.01	0.41 ± 0.11	0.18 ± 0.11
0.1 mgm. histamine	21.8 ± 2.1	0.89 ± 0.13	2.04 ± 0.18	0.67 ± 0.12
3 or 4 cc. yeast extract	32.3 ± 2.9	1.09 ± 0.20	2.09 ± 0.26	2.3 ± 0.5
Second 30 minute period after injection				
0.9 per cent NaCl	5.5 ± 0.9	0.01 ± 0.01	0.29 ± 0.12	0.8
0.1 mgm. histamine	12.0 ± 1.8	0.17 ± 0.05	0.84 ± 0.09	0.9
3 or 4 cc. yeast extract	17.5 ± 3.0	0.61 ± 0.21	1.38 ± 0.31	1.4
Third 30 minute period after injection				
0.9 per cent NaCl	5.0 ± 0.7	0.04 ± 0.02	0.70 ± 0.13	1.4
0.1 mgm. histamine	6.3 ± 1.0	0.05 ± 0.03	0.43 ± 0.24	1.1
3 or 4 cc. yeast extract	10.0 ± 2.0	0.18 ± 0.09	1.00 ± 0.35	1.7

since some output of pepsin is found after injection of 0.9 per cent sodium chloride.

In 6 experiments with these same dogs the injection of 4.5 mgm. of thiamine chloride failed to elicit any more gastric response than followed the injection of 0.9 per cent sodium chloride.

In an effort to determine whether the acid-stimulating factor in our yeast extracts was histamine, both blood pressure effects and chemical behavior have been tested, as follows, but with inconclusive results:

Into an ether anesthetized cat was injected intravenously 0.1 cc. of yeast extract diluted to 1.0 cc. with 0.9 per cent sodium chloride. The resulting

decrease in blood pressure was approximately the same as followed the injection of 0.005 mgm. of histamine in 1.0 cc. of 0.9 per cent sodium chloride. However, after 12 mgm. of atropine, effect of yeast extract had been practically abolished, whereas the histamine depressor effect was still considerable.[5]

When treated with the Pauly reagent by the method of Jorpes (1932) 1.0 cc. of the yeast extract develops a color whose absorption at 495 mμ in the spectrophotometer is equivalent to that of 0.03 mgm. of histamine under like conditions. Whereas the color developed by histamine absorbs maximally at 495 mμ, the yeast extract coloration absorbs much more strongly at 485 mμ.

It may be noted that Lautenschläger (1938) in a review of the industrial significance of yeast has reported the laboratory preparation of histamine from the proteins of yeast and from the products of their decomposition.

SUMMARY

A concentrated extract of fresh yeast has been prepared which, when injected intramuscularly, stimulates gastric secretion in a histamine-like manner. That quantity of extract representing 30 to 40 grams of yeast has an activity equivalent to 0.1 mgm. of histamine. Injection of 4.5 mgm. of thiamine chloride was without effect. While such tests as have been made suggest that the active principle of the yeast extract is not histamine, the evidence on this point must be considered as inconclusive.

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[5] We are indebted to Prof. O. S. Gibbs, of the Department of Pharmacology, for assistance in this assay.

THE EFFECT OF HIGH PRESSURE TREATMENT ON THE PHYSIOLOGICAL ACTIVITY OF INSULIN

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Many of the chemical and physical properties that affect the physiological activity of insulin have been summarized by Jensen (1). The writers are not aware, however, of any study of the effect of hydrostatic pressure on the physiological activity. Several researches have shown that high pressure is an effective means of controlling many reactions of biological and bio-chemical interest. Bridgman (2) in 1914 reported that egg albumin is coagulated by high pressure and with Conant (3) in 1929 found that carboxyhemoglobin is denatured under similar conditions. More recently, Bassett and his collaborators (4) have reported the effects of high pressure on several bacteria, vaccines, and enzymes but the data are of a preliminary nature and few conclusions can be made. Investigations in the high pressure laboratory of the Department of Physics of this College by Dow and Matthews (5) and by Matthews, Dow and Anderson (6) have demonstrated that denaturation of proteins by high pressure results in their deactivation. The extent of denaturation depends on the magnitude of the pressure and the time of exposure, as well as on several chemical factors, for example, the pH of the solution.

Since it is generally agreed that insulin is a typical protein, it would be of interest to study its denaturation at high pressure in relation to its physiological activity.

EXPERIMENTAL METHOD. Insulin solutions from two sources were used in these experiments. Lilly's U-20 Iletin was used in the major part of the investigations and some study was made with amorphous insulin (17 units per mgm.) from the Banting Institute.

The apparatus for hydrostatic pressure treatment is similar to that used extensively by Bridgman (7) and by the senior author in investigating various physical and chemical behavior at high pressure. Initial pressures produced by a hand-pump are further intensified by a hydraulic press that generates high pressure in the test chamber. Pressure can be increased or reduced at such a slow rate that there is no sensible change of temperature during the process. A thermostated bath can be placed about the test chamber to keep it at constant temperature, although in these experi-

ments it was not considered essential and the treatments were applied at room temperature.

The sample of insulin was placed in one of two types of containers. Flexible tooth paste tubes lined with paraffin were first tried as containers. The tubes were used by soldering the lower ends, filling with the test sample, and then sealing by means of the threaded cap. Consequently the sample was separated mechanically from the kerosene that transmitted pressure in the test chamber. A brass tube, lined similarly with paraffin and closed at one end, served as the other type of container. As the samples did not completely fill the tube, paraffin oil was poured in to cover the insulin with a layer of about one inch in depth. Other experiments had shown previously that good separation of liquids was obtained in this way and that similar results were found irrespective of the nature of the container. This is similar to the experience of Basset (4). Both containers were used indiscriminately in these experiments but most of the tests were made with the latter type.

The physiological activity of both normal and pressure treated insulin was measured by the percentage lowering of blood sugar in rabbits that were given standard doses, and doses of the pressure treated samples that were considered the equivalent of standard doses. A standard solution was considered to have 2.5 physiological units per cubic centimeter of solution and was made up with U-20 Iletin in physiological salt solution. A standard dose was taken as 1 cc. of solution that contained 2.5 units per 2 kgm. of body weight. The pressure treated samples were made up in the same way by using the same quantity of treated insulin in physiological salt solution. For the pressure treatment, however, the Iletin was used as it was received from the maker but the amorphous insulin was made up in physiological salt solution to contain 140.1 units per cubic centimeter. While the onset of convulsions, as well as the intensity and length of them, were observed they were not considered sufficiently reliable measure of the insulin activity to be recorded in this paper. One reason for this was the wide variation of the convulsive level in the rabbits.

The animals were not ideal for the purposes of the experiments. They were of mixed breed and evidently varied considerably in physical stamina. Several of their ears were unsuitable for bleeding. Two females and ten males were used in the experimental procedure. Of the former, rabbit 7 unexpectedly gave birth prematurely to a litter during one of the tests. She had been used previously several times, giving consistent behavior as can be judged by the data. Culhane (8) has reported that females having a litter are generally unsuited for tests. It is thus surprising to observe such consistent results with a pregnant rabbit. The ears of the other female were so unsuited for bleeding that she could not be used after a preliminary experiment.

The method of "cross-test" advocated by Culhane (8) was used in the

experiments. By this procedure one group of animals is given the standard preparation and another group the test sample. This method was followed twice a week using four animals for each test but each animal was not used more than once a week in most cases. Two rabbits were usually given the standard insulin dose and two others the pressure treated sample. The tests were carried out over a period of five weeks.

The diet prescribed for the rabbits consisted of hay and oats. The rabbits were without food 24 hours prior to the experiments. During this period, as well as during the tests, they were allowed an ample amount

TABLE 1

Percentage sugar decrease in rabbits inoculated with pressure treated and untreated iletin

ANIMAL	PER CENT CHANGE UNTREATED ILETIN	PER CENT CHANGE WITH PRESSURE TREATED ILETIN					AVERAGE PER CENT CHANGE OF PRESSURE TREATED ILETIN	PER CENT DIFFERENCE TREATED-UNTREATED ILETIN
		1 5,000 kgm./cm².	2 5,000 kgm./cm².	3 10,000 kgm./cm².	4 10,000 kgm./cm².	5 10,000 kgm./cm².		
1	45		30	17	46	43	32	−13
2	No results							
3	71	71	73 Died				72	+1
4	47		31	36			34	−13
5	55*	65	74	51	Died		63	+8
6	56		51	43	39		44	−12
7	55*		59	45	47		50	−5
8	57*			38 53**	64	57	53	−4
9	60	64	66	40	69	59	60	0
10	58	70	77 Died				74	16
11	63				46	62	54	−9
12	No results							
Average								−5

* Two determinations differing by 1%.

** Amorphous insulin treated for 3 hrs.

of water. After inoculation samples of blood were drawn from the ears at 1½, 3 and 5 hours, respectively. These together with the blood sample that was always taken before inoculation were sufficient to allow the change in blood sugar to be followed with some accuracy. The micromethod of Folin and Malmros (9) that requires only 0.1 cc. of blood was used to determine the blood sugar content.

DATA AND RESULTS. The experimental results summarized in table 1 were obtained with Iletin, except in one case as indicated. The maximum percentage decrease in blood sugar level is recorded for normal insulin and

pressure treated samples at 5,000 and 10,000 kgm/cm². Two insulin samples were treated at 5,000 kgm/cm². The exposures, tests 1 and 2, were for 3 and 15 hours, respectively. Three treatments at 10,000 kgm/cm², tests 3, 4 and 5, were made for 15, 23 and 41 hours, respectively. To determine the maximum percentage decrease in sugar level, the four sugar determinations, measured as the number of milligrams per 100 cc. of blood, were plotted graphically and the maximum decrease was read from the resulting curve in each case. This method was adopted since it was found that the curves were always similar but not of a simple nature. Since they showed a definite relation in respect to time, a simple average of sugar determinations during the 5 hour period would have little significance.

While it can be shown that the average of the percentage decreases for the treated samples is about 5 per cent less than the average for untreated insulin, this difference does not prove necessarily that the physiological activity of insulin is reduced by pressure treatment. Considering that the animals used in this study were not the best for the purpose, and the fact that relatively few were available for the tests, it would appear reasonable to assume that 5 per cent represents a likely error in these experiments. On this basis the writers are inclined to state that pressure treatment had no effect on the physiological activity of insulin.

Although the pressure treatments did not change the appearance of Iletin, it was realized that with stronger concentrations of insulin, and particularly with insulin that contained no preservative, pressure might produce coagulation as had been observed previously (6) in the case of pepsin. To verify this the amorphous insulin in physiological salt solution was subjected to a pressure treatment of 10,000 kgm/cm² for 3 hours. In this case, however, the treatment did produce coagulation that was clearly visible. The precipitate appeared similar to the appearance of egg albumin, or any other typical protein, such as pepsin, when it is coagulated by heat. The precipitate was carefully mixed with additional salt solution to a dilution corresponding to the previous doses and then injected into a starved rabbit. The animal was no. 8 that had been used in the previous experiments. The maximum percentage decrease in sugar was found to be 53 per cent, a value close to that found for the animal previously (table 1). The physiological activity of amorphous insulin was clearly not affected by the fact that coagulation resulted from the pressure treatment.

Van Slyke amino nitrogen determinations were made on both the control solution of amorphous insulin and the pressure treated sample with the result that no change in amino nitrogen was detected. Consequently there is no evidence of hydrolysis by pressure.

Discussion. It is well known that insulin loses its physiological

potency when denatured by heat. In this respect it behaves similarly to many other proteins. The present experiments show, however, that while insulin may be denatured by pressure, as indicated by coagulation in concentrated solutions, the treatment does not reduce its physiological activity. Evidently denaturation caused by pressure must be fundamentally different from that brought about by heat. Since when applying pressure to a sample the rate of compression can be so low that there is no appreciable rise of temperature, the process can be said to be carried out isothermally. It is important to note that during such a compression more energy flows out of the sample in the form of heat than is put in by the work of compression. This is easily understood when it is recalled that the attractive forces in the liquid must do work during the compression. It has been shown previously (6) that by compressing water isothermally to a pressure of 1,000 kgm/cm.^{2}, about seven times more energy flows out of the water than is put in by the compression. When it is remembered that in denaturing by heat energy must always flow into the sample, as indicated by the temperature rise, it is evident that in pressure denaturation the physical process insofar as energy is concerned, is decidedly different. This appears to be a significant factor that has not been studied thoroughly. It suggests many possibilities for future study since it may involve some reaction within the protein molecule that is not known at present.

The recent theory of Wrinch (10, 11, 12) on the structure of proteins has given impetus to speculations on the nature of the changes that take place in denaturation and hydrolysis. According to this theory denaturation takes place when the cyclol bonds are broken, but the polypeptide chains remain uneffected. A breaking of a peptide link, however, involves a reaction with another molecule and is to be associated with hydrolysis. According to this interpretation, the pressure denaturation observed in these experiments broke the cyclol bonds in the insulin molecules with the result that the polypeptide chains became the predominating type of structure. No change in amino nitrogen would be expected unless hydrolysis occurred. Since no change of activity has been observed in these experiments, it would appear that the physiological properties of insulin are bound up in some manner with the polypeptide linkage in the molecule, denaturated or otherwise. The possibility of associating the specific physiological properties of insulin with its component amino acids has been discussed by Jensen (13).

The authors wish to acknowledge the donation of the Iletin by Dr. W. B. Peck of the Lilly Research Laboratories and the amorphous insulin by Professor W. R. Franks, of the Banting Institute. They are indebted to Dr. A. K. Anderson of this college for his kindness in putting his equipment at their disposal.

SUMMARY

The physiological activity of insulin is unaffected by long exposure to pressure of the magnitude of 10,000 kgm/cm^2. Activity was measured by observing the percentage decrease of blood sugar level in rabbits.

Evidence that pressure produced denaturation is given by the coagulation of insulin. Coagulation does not affect the physiological activity. The absence of any change in amino nitrogen content of the insulin has been taken to mean that pressure does not produce hydrolysis under these conditions.

It is suggested that the results of these experiments indicate that the physiological activity of insulin is associated with its polypeptide or amino acid linkages, rather than with any particular bonding or grouping of the chains such as might exist in the undenaturated state of the molecule.

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DEHYDROGENASE INACTIVATION IN OXYGEN POISONING[1]

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Libbrecht and Massart (1937), using the Warburg technique, demonstrated a decreased oxygen uptake of a succino-dehydrogenase system exposed to oxygen at high pressures. They concluded that the high concentrations of oxygen inactivated the dehydrogenase system. We have been interested in the possible rôle this dehydrogenase inactivation might play in the induction of the toxic effects of oxygen at increased barometric pressure on various tissues. Such toxic effects are exemplified by a decrease in the strength of contraction of striated muscle (Bean and Bohr, 1938), an upset in the pace setting mechanism of the isolated heart (Bohr and Bean, 1939), and a fall in the tonus and loss of rhythmicity of isolated non-striated muscle (Bean and Bohr, 1940). In each of these tissues the return to oxygen at atmospheric pressure is attended by a complete or partial recovery of normal function, the degree of recovery being conditioned by the severity and duration of the exposure to increased pressure. Where the tissue is exposed a second time these deleterious effects have a more rapid onset, are more pronounced and recovery—if any—invariably incomplete.

In our attempt to determine whether the influence of oxygen at high barometric pressure on isolated tissue might possibly be related to an inactivation of dehydrogenase, the Thunberg methylene blue reaction (Thunberg, 1920) was used as an index of dehydrogenase extract activity. The changes of such activity induced by exposure of the extract to oxygen at increased barometric pressure of various intensities and durations, were followed, as well as the possible recovery of the activity after the return of the extract to oxygen at atmospheric pressure.

In our experiments a dehydrogenase-cytochrene system was prepared following the method of Stotz and Hastings (1937), but pork hearts, weighing close to 200 grams, were used instead of beef hearts. The extraction was carried out on the day of the killing, and the extract was preserved by overlaying with toluene and storage in an icebox at 5°C. Although there was no significant decrease in activity of the enzyme solution preserved

[1] These experiments were supported by a grant from the Rockefeller Foundation to Robert Gesell for studies on respiration.

in this manner after a week's storage, all of the experiments referred to in this report were carried out within 48 hours after the preparation of the extract. This precaution was taken in view of the rapidly deleterious effect of ageing on the oxygen activating systems, and the important part that this system is said to play in the inactivation of dehydrogenase by high oxygen (Libbrecht and Massart, 1937).

A tonometer rotated by a small electric motor mounted inside of the compression chamber was used to equilibrate the extract with the desired pressure of oxygen. Rapid equilibration was assured by using only a small volume of the extract (10 cc.) in a large tonometer (200 cc.). The temperature of the chamber was maintained at 37°C. and the oxygen used in compression was saturated with water to prevent concentration of the extract. Similar methods were used to equilibrate the extract with the pure nitrogen or air used as control gases.

In preliminary experiments we found that vigorous mechanical agitation as produced by bubbling the extract solution with gas caused a decrease in dehydrogenase activity. That such a decrease in activity during bubbling was due to mechanical agitation rather than to any peculiarity of the gas employed, was shown by the fact that it occurred as a result of bubbling, not only with oxygen, but also with air and pure nitrogen. The degree of the inactivation of the dehydrogenase so induced is apparently dependent upon the violence and duration of the agitation, for it was found that if equilibration of the extract with air or pure nitrogen was accomplished by very gentle rotation of the tonometer, no diminution in the activity of dehydrogenase occurred. In light of these findings precautions to eliminate so far as possible any mechanical agitation of the extract assume great importance. In all of our experiments, therefore, equilibration of the extract was accomplished by gentle rotation of the tonometer, not only during exposure to increased pressure, but also during decompression to facilitate the escape of dissolved gas without the formation of bubbles in the fluid.

Immediately after a given exposure to oxygen at increased barometric pressure or to the control gas, and before the methylene blue reaction mixture was made up, the extract was placed in a 250 cc. flask and evacuated just to the boiling point at 37°C. This negative pressure was maintained for a period not longer than 1 minute, during which time the flask was gently swirled to promote rapid liberation of oxygen from the solution. In this manner the oxygen tensions of the test and control extracts were adjusted to the same level. Such deoxygenation is essential since oxygen, if left in solution, will itself act as a hydrogen acceptor and thus cause an increase in the reduction time of methylene blue. To eliminate the possible involvement of the influence of mechanical agitation on the dehydrogenase activity in this procedure, the precautions against violent

and prolonged agitation as mentioned above were rigidly observed. It was demonstrated that this method of deoxygenation did not alter the dehydrogenase activity.

The reaction mixture used in testing the activity of the dehydrogenase factor after a given exposure to the gas was essentially similar to the standard reaction mixture as used by Lehman (1930). The dehydrogenase extract was diluted (with a $\frac{1}{15}$ M. NaH_2PO_4-Na_2HPO_4 buffer, pH 7.29) in order to give a methylene blue decolorization time between 10 and 15 minutes. The Thunberg tubes, containing the reaction mixtures indicated in the tables below, were placed in a 37°C. water bath and evacuated with a "Cenco-Hyvac" vacuum pump. The time for decolorization of methylene blue was taken from the moment boiling commenced.

TABLE 1

Heart A

TUBE NO.	TUBE CONTENT				METHYLENE BLUE REDUCTION TIME
	0.0004 M. meth. blue	0.2 M. succinate	Control extract (air at atm. pressure)	Test extract (O_2 at 7.6 atms. pressure)	
	cc.	*cc.*	*cc.*	*cc.*	*min.*
1	0.3	0.2	2.5		12
2	0.3	0.2		2.5	19.5
3	0.3	0.2	2.5		12
4	0.3	0.2		2.5	20.5
5	0.3	0.2	2.5		12
6	0.3	0.2		2.5	19.5

The first table demonstrates a typical alteration in activity of the succino-dehydrogenase system produced by exposure to oxygen at 100 pounds' pressure (7.6 atmospheres).

In this series, exposure of the test extract (tubes 2, 4, and 6) to oxygen at 100 pounds' pressure (7.6 atms.) for one and one-half hours caused a 40 per cent inactivation of the dehydrogenase as measured in methylene blue reduction time.

It is noteworthy that the degree of inactivation produced by high oxygen varied from one preparation to the next. For example, in extracts of four different hearts inactivations of 50 per cent, 42 per cent, 9 per cent and 20 per cent were produced by two and one-half hours' exposures to oxygen at 100 pounds' pressure. Yet repeated exposures of extracts from the same heart gave consistent degrees of inactivation. This variation between extracts from different hearts is of interest in view of the striking differences in susceptibility of intact animals to oxygen poisoning. Such differences have been attributed by some investigators to the age (Massart, 1936), the state of nutrition (de Almeida, 1934), and the thyroid gland activity

(Campbell, 1938) of the animal. Youth, starvation and thyroidectomy are said to be of protective value against oxygen poisoning. The inconstancy in the susceptibility of the dehydrogenase extracts from different animals to inactivation by oxygen at high barometric pressure as shown in our experiments may represent an additional basis for the differences in the susceptibility of the intact animal to oxygen poisoning. On the other hand, it is conceivable that the differences explained as due to age, nutrition and thyroid activity are in reality due to corresponding conditions of the dehydrogenase system or its environment which alter its susceptibility to oxygen at high pressure. For example, the dehydrogenase system which governs a particular reaction in the metabolism of the growing cell (i.e., in youth) might be expected to differ from that system in the mature cell and it may be that the enzyme during the metabolism of growth is

TABLE 2

Heart A

TUBE NO.	TUBE CONTENT					METHYLENE BLUE REDUCTION TIME
	0.0004 M. meth. blue	0.2 M. succinate	Control (fresh from icebox)	O_2 atm. pressure	7.6 atms. pressure (N_2)	
	cc.	*cc.*	*cc.*		*cc.*	*min.*
1	0.3	0.2		2.5		13
2	0.3	0.2	2.5			13
3	0.3	0.2			2.5	13.5
4	0.3	0.2		2.5		13
5	0.3	0.2	2.5			13
6	0.3	0.2			2.5	13.5
7	0.3	0.2		2.5		13
8	0.3	0.2	2.5			13

more resistant to inactivation by high oxygen pressure than the enzyme of the mature cell. A study of the resistance of dehydrogenase systems from animals of different age groups would be of interest in this connection.

With the purpose of determining the effect of oxygen at atmospheric pressure and of 100 pounds' pressure *per se* on the activities of the dehydrogenase extract the following experiment was carried out.

The extract used in this experiment was taken from the same heart as was that used in the first experiment—the results of which are shown in table 1. The "control" extract of the above résumé (table 2) was kept in the icebox, while the other two were equilibrated in tonometers for one and one-half hours—one with oxygen at atmospheric pressure, and the other with nitrogen at 100 pounds' pressure (7.6 atms.). These control measures did not cause an appreciable alteration in dehydrogenase activity.

As noted above, isolated tissues suffering from the deleterious effects of high oxygen pressure frequently regained their normal function when the

oxygen concentration was reduced to atmospheric pressure. Such recovery was usually maximal within one-half hour after decompression to atmospheric pressure. In order to determine whether this recovery of normal tissue activity might not be explained on the basis of a concomitant recovery of dehydrogenase activity, the following experiment was performed: After the activity of the extract had been diminished by exposure to oxygen at 100 pounds' pressure (7.6 atms.) for one hour, the pressure was reduced to atmospheric; the extract evacuated and gently swirled and its activity determined at fifteen minutes and at one hour following the decompression. The results of such determinations are summarized in table 3 below.

TABLE 3

Heart B

TUBE NO.	TUBE CONTENT				METHYLENE BLUE REDUCTION TIME
	0.0004 M. meth. blue	0.2 M. succinate	Air control extract atm. pressure	Extract exposed to O_2 at 7.6 atms.	
	15 minutes after decompression				
	cc.	*cc.*			*min.*
1	0.3	0.2	2.5		7
2	0.3	0.2		2.5	9.5
3	0.3	0.2	2.5		7.5
4	0.3	0.2		2.5	9
5	0.3	0.2	2.5		7.5
	One hour after decompression				
1	0.3	0.2	2.5		7
2	0.3	0.2		2.5	9.8
3	0.3	0.2	2.5		7
4	0.3	0.2		2.5	9
5	0.3	0.2	2.5		7

Even one hour after decompression from an exposure to oxygen at 100 pounds' pressure the test extract (tubes 2 and 4) showed no signs of spontaneous recovery. Several explanations might be offered for this lack of parallelism between the apparent recovery seen in isolated tissues following decompression from high oxygen and the absence of recovery in the dehydrogenase system *in vitro*. It may be that either some element capable of reactivating the dehydrogenase system present in isolated tissue is missing in the extract preparations, or that the reversible decrease of normal function of the isolated tissue exposed to high oxygen is based on some reversible process which does not involve the dehydrogenase system. A third possibility presents itself when we consider the fact that

although the isolated tissues seem to have regained normal functional activity after an initial exposure to high oxygen, a second exposure causes a more rapid onset of the deleterious effects, the recovery from which is usually less than complete. Thus, in spite of the apparent return to normal function after the first decompression, there must be some mechanism that has not shared such recovery. It is here that the irreversible inactivation of this dehydrogenase system may fit into the picture of oxygen poisoning.

SUMMARY

Experiments are described in which the effects of oxygen at high barometric pressure (7.6 atms.) on succino-dehydrogenase extracts of pork hearts were determined.

The importance of elimination of violent or prolonged mechanical agitation of the extract, such as that of bubbling during its equilibration with the overlying gas, is stressed since it was found that such agitation in itself may alter dehydrogenase activity.

Exposure of the extracts from different hearts to oxygen at 100 pounds' pressure (7.6 atms.) for two and one-half hours resulted in decreased activity of the enzyme of from 9 per cent to 50 per cent. It is suggested that the variability in degree of inactivation might be one underlying cause for the lack of uniformity in susceptibility of intact animals to oxygen poisoning.

The inactivation of the dehydrogenase system in these *in vitro* experiments appears to be an irreversible process. The relationship between this apparent irreversible inactivation of dehydrogenase extract and the degree of functional recovery which occurs in isolated tissue preparations following exposure to high pressures of oxygen is discussed.

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DIFFERENTIAL SENSITIZATION OF ADRENERGIC NEURO-EFFECTOR SYSTEMS BY THYROID HORMONE[1]

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Sensitization of the sympathetic nervous system to epinephrine is a common explanation for various signs and symptoms encountered in hyperthyroid states (19). Among the symptoms of hyperthyroidism which would be explained at least in part if adrenergically innervated effectors were sensitized to adrenalin by thyroid hormone are functional exophthalmos, widened lid-slits, von Graefe's sign, dilated pupil, tachycardia, the Goetsch epinephrine test, and possibly some of the disturbances in carbohydrate metabolism. On the other hand, certain fairly common symptoms of thyrotoxicosis are the reverse of what would be expected if all adrenergically innervated effectors were sensitized. Included in these latter symptoms are diarrhea, excessive hunger, and dilatation of skin vessels. Means (10) has indicated that virtually all of the symptoms of hyperthyroidism could be produced by hypermetabolism *per se*. This being the case, postulation of sensitization of the sympathetic nervous system would be unnecessary.

The sympathetic system includes at least four physiological types of neuro-effector systems, namely: excitatory adrenergic (EA), inhibitory adrenergic (IA), excitatory cholinergic (EC), and inhibitory cholinergic (IC). A review of the extensive literature on the thyroid-autonomic relationship reveals that, with few exceptions, studies of sensitization of sympathetically innervated effectors to chemical mediators by thyroid hormone have been concerned with sensitization to adrenalin in the EA type of system. The most frequently used indicators have been the blood pressure, the caliber of skin vessels, and the heart rate. Although opposite results have been obtained, the majority of these studies indicate that in thyrotoxic animals certain effectors receiving an excitatory adrenergic innervation show a hypersensitivity to adrenalin. Some of the literature on the effect of thyroid hormone on the heart rate will be cited since the S-A node was used as an indicator in this study.

McIntyre (9) has reported an essentially normal degree of acceleration of the denervated dog heart as a result of thyroid feeding. A direct

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accelerator effect of thyroxin is also indicated by persistence of the fast rate in the denervated (9) or in the isolated (6) thyrotoxic heart. In the latter experiments epinephrine was no more effective in the thyroxinized, isolated hearts than in the normal. A greater than normal accelerator response to adrenalin in chronically thyrotoxic hearts has been reported for cats (14), frogs (4), and terrapins (8). In electrocardiographic studies Rosenblum et al. (13) found the thyrotoxic rabbit heart more susceptible than normal hearts to the irregularity-producing effects of adrenalin. Administration of thyroid substance to man exaggerates the cardiovascular reaction to adrenalin (7). The above studies show that a considerable part of the tachycardia observed in an intact thyrotoxic animal is explicable on the bases of increased demands on the heart accompanying the rise in total metabolism and a direct stimulatory action of thyroid hormone on the heart. Heightened sensitivity to epinephrine and to the adrenergic mediator appears to be a third factor which would tend to increase the lability of the accelerator mechanism rather than exert much influence on the basal rate.

Apparently no study has been made to determine if *inhibitory* adrenergic neuro-effector systems in intact animals are sensitized by thyroid hormone. However, various facts are suggestive. The smooth muscle of the non-sphincteric parts of the stomach and intestine receives an inhibitory adrenergic innervation from the sympathetic nervous system (20), (23), (24). Clinical observations indicate that the motility of the gastro-intestinal tract tends to be reduced in hypothyroidism (1), (18) and increased in hyperthyroidism (15), (16). Fetter and Carlson (3) found that hyperthyroidism stimulated gastro-intestinal motility in experimental animals, and they cited previous literature supporting this view. Recently Morrison and Feldman (11) have reported that induced hyperthyroidism increases gastro-intestinal motility with or without sectioning of the vagus nerve. Such facts argue against but do not disprove sensitization of tonically active inhibitory adrenergic intestinal nerves by thyroxin.

Since adrenalin is considered to be the mediator produced at adrenergic nerve endings, this substance should be more inhibitory to the intestine of thyrotoxic animals than of normal animals if thyroid hormone sensitizes the entire sympathetic nervous system. Experiments on the isolated rabbit intestine have failed to show any sensitization to the inhibitory effects of adrenalin by thyroid hormone (5) (12). The experiments described below show that the dog intestine "in situ" undergoes no increased sensitivity to adrenalin during a period of thyroid feeding which produces the characteristic cardiac sensitization.

METHODS. Records of the following were obtained from each of five dogs before commencement of thyroid feeding. 1. The heart rate under near-basal conditions. 2. Minimal cardio-accelerator dose of adrenalin.

and the effects on heart rate of doses two to four times as great. 3. Motility of a Thiry fistula of the jejunum. 4. Minimal intestine-inhibiting injection rate of adrenalin, and inhibitory effect of doses two to four times as great. 5. Degree of reflex inhibition of the intestine by adrenergic nerves during rectal stimulation. 6. The intestinal responses to the sympathomimetic substances reflexly liberated during acetylcholine hypotension. Records of heart rate were taken with an electrocardiograph, and records of intestinal motility were taken by a balloon-mercury-manometer system. After completion of the above analysis of the sensitivity of the sino-auricular node (EA innervation) and of intestinal smooth muscle (IA innervation) to injected and reflexly liberated sympathomimetic substances, the animals were fed 0.8 to 1.2 grams of powdered thyroid (Armour & Co.) per kilo daily for a period of two to five weeks. Procedures 1 to 6 were then duplicated in the thyrotoxic animals to evaluate alterations in sensitivity of the two types of adrenergic neuro-effector systems.

Results. 1. *Effect of thyroid hormone on the sensitivity of the cardio-accelerator mechanism (EA) to adrenalin.* Four of the five thyroid-fed dogs developed the general signs indicative of thyrotoxicosis. These consisted of warm skin, high basal heart rates, hyper-excitability, excessive cardiac acceleration and panting with mild exercise. One of the five dogs developed diarrhea and failed to show signs of thyrotoxicosis, presumably because of insufficient absorption of thyroid hormone from the intestine. The following results were obtained from the four remaining dogs.

a. *Effect of thyroid hormone on the basal heart rate.* Each of the four animals showed an increase of approximately 30 to 60 beats per minute in the basal heart rate during the course of the feeding of thyroid substance. The average basal rate before thyroid feeding was 112 as compared with 161 after two to six weeks of feeding. It was more difficult to obtain basal heart rates in the latter case because of greater excitability of the animals and greater accelerator response to mild exercise. One of the animals was particularly well-trained, having been used for similar experiments under near-basal conditions for four years.

b. *Responses to continuous injection of adrenalin at low rates.* The average response of the normal dogs to a continuous intravenous injection of adrenalin, 1 part in 500,000, at a rate of 1 cc. per minute is illustrated in figure 1. The heart rate, after a latent period, was accelerated 17 beats per minute. This injection rate is recognized as being barely threshold for production of circulatory effects in the normal unanesthetized dog (21). The same injection rate in the same animals after 2 to 5 weeks of thyroid feeding resulted in a rate increase of 44 beats per minute over the already high basal rate. At the end of 75 seconds of injection of adrenalin an average rate of 124 beats per minute was obtained in the animals before

thyrotoxicosis as compared with a rate of 201 for the same animals after thyroid feeding.

Comparison of figures 1 and 2 will illustrate the differences in responses obtained when the adrenalin injection rate was doubled or quadrupled. Reflex inhibition of the heart as a result of the pressor effects of the higher dosage (fig. 2) becomes a factor but the reflex inhibition is less effective in opposing the direct cardio-accelerator action of the adrenalin in the thyro-

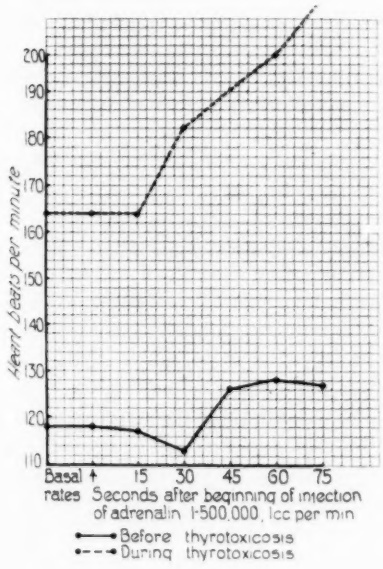

Fig. 1

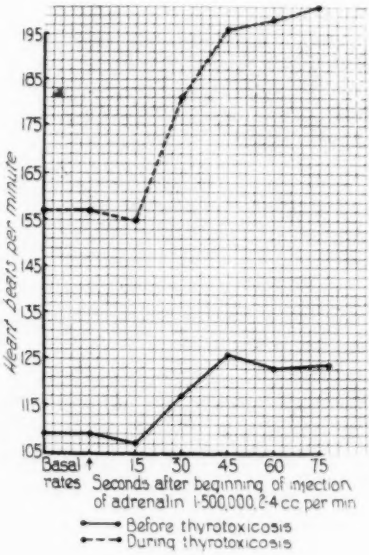

Fig. 2

Fig. 1. Average effect of continuous intravenous injection of adrenalin, 1 part in 500,000, at a rate of 1 cc. per minute on the heart rate of a series of dogs before and during thyrotoxicosis produced by feeding powdered thyroid.

Fig. 2. Average effect of continuous intravenous injection of adrenalin, 1 part in 500,000, at rates of 2 and 4 cc. per minute on the heart rate of a series of dogs before and during thyrotoxicosis.

toxic state than in the normal. It is to be expected that the pressor responses resulting from a given dose of adrenalin after thyrotoxicosis should be at least as great as in the same animals before thyrotoxicosis (2). Reflex inhibition of the heart quickly opposes the direct accelerator action of adrenalin, 1-500,000, injected at rates of 2 to 4 cc. per minute in the normal dogs so that the heart rate rises only 15 beats per minute above the basal rate, but the thyrotoxic hearts show an increase in rate to approximately 48 beats per minute above the high basal rate at the end of 75

seconds. If the pressor response to this amount of adrenalin during thyrotoxicosis is not less than before the thyroid feeding, the greater accelerator action of adrenalin in the thyrotoxic animals may be interpreted as indicating either sensitization of the cardio-accelerator mechanism or subnormal effectiveness of the cardio-inhibitory mechanisms. Heightened sensitivity to the accelerator action of adrenalin in isolated thyrotoxic hearts supports the interpretation that peripheral sensitization of the cardio-accelerator mechanism is, at least in part, responsible for the results obtained. Moreover, it has been reported (17) that thyroxin increases the effectiveness of the cardio-inhibitory mechanism.

2. *Effect of thyroid hormone on the sensitivity of intestinal smooth muscle (IA) to adrenalin, adrenine, and adrenergic neurohormones.* a. *Responses to injected adrenalin.* The five dogs were given 41 injections of adrenalin at rates ranging from slightly below the threshold intestine-inhibiting dose to doses four times as great. The records of intestinal motility were obtained from innervated Thiry fistulae in each of the animals. Simultaneous records were obtained from innervated and denervated Thiry fistulae in one of the animals. After having determined the sensitivity of the intestine of each normal animal to adrenalin, thyroid feeding was begun. A total of 47 adrenalin injections, duplicating the rates before thyroid feeding, were given the animals after the appearance of thyrotoxicosis was indicated by the general signs and the specific cardiac sensitization to adrenalin described above. Neither the innervated nor the denervated intestine of the thyrotoxic dogs showed any hypersensitivity to the inhibitory effects of adrenalin. The responses of the intestine of one of the dogs to adrenalin before and during the period of thyroid feeding is illustrated in figure 3. If thyroid hormone has any effect on the intestine-inhibiting potency of adrenalin it decreases it.

b. *Responses to reflexly liberated adrenine.* The brief fall in blood pressure caused by intravenous injection of a selected dose of acetylcholine into unanesthetized dogs produces a sharp fall in blood pressure which causes reflex liberation of adrenine from the adrenal medulla (22). The acetylcholine itself is quickly destroyed so that, after a few seconds, the effects of adrenine on denervated indicators may be observed. The adrenine-inhibition of the intestine produced following acetylcholine injection is no greater in the thyroid-fed dogs than in the same animals before feeding thyroid.

c. *Effect of thyrotoxicosis on the sensitivity of intestine-inhibiting reflexes.* Rectal stimulation reflexly activates inhibitory adrenergic nerves to the intestine causing, in sensitive unanesthetized dogs, complete inhibition of intestinal motility and entrance of sympathin into circulation (23). After a latent period a typical adrenalin effect is observed on the motility of a denervated intestinal segment in an adrenal demedullated, vagoto-

mized animal (23). Since the mediator of impulses at adrenergic nerve endings is apparently identical to adrenalin, in view of the facts presented in

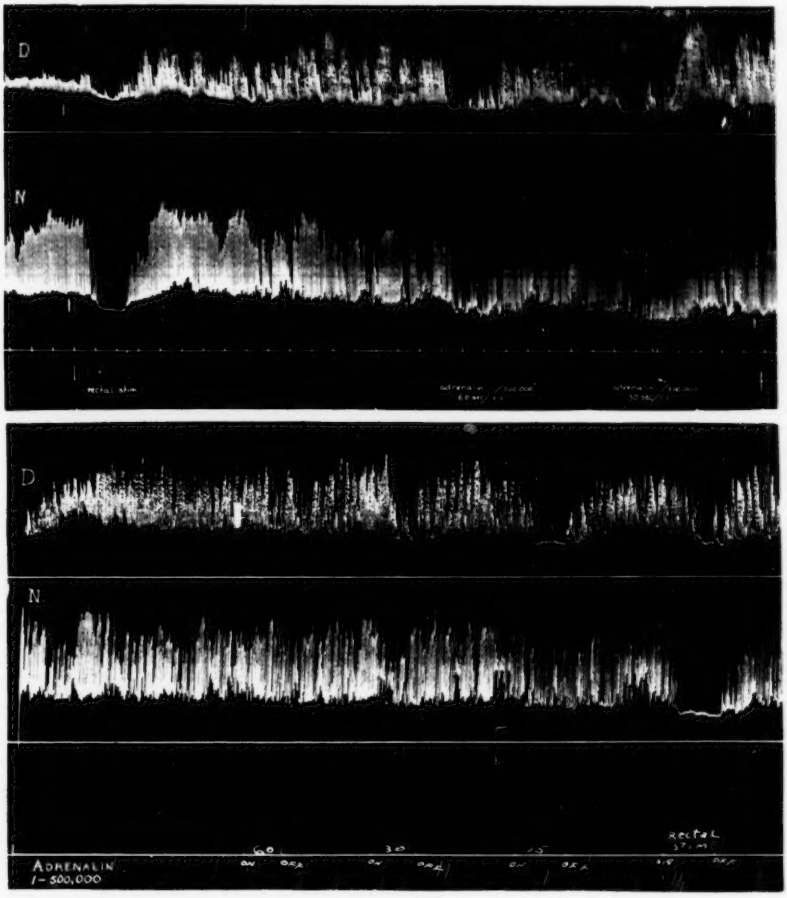

Fig. 3. *Upper record.* Effect on the motility of the denervated (D) and innervated (N) intestinal segments of rectal stimulation, injection of adrenalin, 1 part in 500,000, at a rate of 1 cc. per minute, and 2 cc. per minute. Time in minutes.

Lower record. Records from the intestinal segments of the same dog after four weeks of thyroid feeding. Effect of adrenalin, 1-500,000, at rates of 1, 2 and 4 cc. per minute, and rectal stimulation. Drum speed same as for upper record.

2 a, it is not to be expected that the recto-intestinal reflex would be sensitized during thyrotoxicosis unless the sensitization is elsewhere than

in the effector. Figure 3 illustrates the failure to increase the inhibitory effects of rectal stimulation in either the innervated or denervated intestine by thyroid feeding.

d. *Interpretations.* If alterations in the sensitivity of the intestine to adrenalin occur they are placed in evidence by near-threshold inhibitory injection rates. This method clearly depicts the development of hypersensitivity to adrenalin in the intestine after adrenergic denervation of the intestine (20). Therefore, the results presented in a to c indicate that the thyrotoxic intestine is not sensitized to injected adrenalin, reflexly liberated adrenine, the adrenergic mediator, or "intestinal" sympathin. The latter substances are similar, if not identical, to adrenalin. The absence of sensitization of the adrenergic intestine-inhibiting mechanism is further indicated by the fact that intestinal motility is not reduced by thyroid feeding. Sensitization of this mechanism would result in lowered motility of the intestine if the nerves are tonically active.

The question arises if it may be concluded that all smooth muscle having an IA innervation, whether in the intestine, bronchioles, bladder, nonpregnant uterus, or certain arterioles, is not sensitized to adrenalin by thyroid hormone. The necessity for a physiological classification of smooth muscle has been suggested (21). Such a classification is at present too incomplete to allow generalizations; however, it is likely that the intestine and other dually innervated smooth muscle having IA and EC innervation belong to the same physiological type.

SUMMARY AND CONCLUSIONS

A review of the literature on the relation of thyroid hormone to the sensitivity of autonomic neuro-effector systems reveals the fact that most observations have been concerned with excitatory adrenergic (EA) neuro-effector systems, such as the cardio-accelerator and vasoconstrictor mechanisms. Three other physiological types of autonomic neuro-effector systems, which have been given little attention, are inhibitory adrenergic (IA), excitatory cholinergic (EC), and inhibitory cholinergic (IC). Results obtained in the study of any one of these systems do not afford a basis for generalizations involving the other three.

An experimental analysis of the sensitivity of the cardio-accelerator mechanism (EA) and the intestine-inhibiting mechanism (IA) of dogs under near-basal conditions to adrenalin before and during thyrotoxicosis indicates that marked sensitization of the former mechanism occurs in the absence of any sensitization of the latter. The intestinal smooth muscle of the thyroid-fed animal is not hypersensitive to adrenalin or to adrenergic neurohormones, and there is no increased effectiveness of intestine-inhibiting reflexes utilizing adrenergic nerves.

Explanation of the thyrotoxicosis syndrome in the dog, in addition to the

usual recognition of the responses resulting from increased metabolism *per se* and direct effects of the thyroid hormone on effectors, requires recognition of *differential sensitization* of autonomic neuro-effector systems. The sensitization of these systems can not be stated in terms which refer to anatomical units of the visceral efferent system containing various physiological types of neuro-effector systems. Exactly which systems are sensitized and which are not must be determined by further experimentation.

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STUDIES ON THE RATES OF ABSORPTION OF WATER AND SALTS FROM THE ILEUM OF THE DOG

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It has been found by Roepke and Visscher (1939) that, during absorption from the dog's lower ileum of originally isotonic salt solutions, those solutions become significantly hypotonic as judged by vapor tension measurements. This phenomenon occurred when mixtures of NaCl and Na_2SO_4 in solution underwent absorption. If such solutions were originally slightly hypotonic they remained so, and if they were not too greatly hypertonic their osmotic activity decreased to less than that of the animal's blood. The same authors showed that when autogenous serum underwent absorption its apparent osmotic activity decreased to the extent of a half atmosphere osmotic pressure during the process. These observations point to a net absorption of solute in excess of its isotonic equivalent of solvent. The experiments using serum are particularly important because originally there are no concentration differences whatever, and any hydrolytic enzymatic processes occurring would increase rather than decrease osmotic activity. Studies have been made of the net movement of water and salt from various salt mixtures in order to ascertain the relation between the two. Such observations are reported in this communication.

From previous studies it has become apparent that the presence of polyvalent ions greatly influences the rate of uni-univalent salt absorption. No observations have as yet been reported, however, in which the rates of absorption from isotonic solutions containing various proportions of univalent and polyvalent ion salts have been studied under standardized conditions. In view of the difficulty of maintaining adequate constancy of conditions in repeated absorption studies in acute experiments under anesthesia, observations have been made upon dogs with chronic ileal loops. With suitable precautions repeated observations on such loops give constant results and comparisons can be made of the absorption rates under the several conditions.

Rates of water absorption over time are difficult of measurement by simple volume studies because it is impossible to empty the intestine completely several times during a single absorption period by feasible mechan-

ical means, and because the trauma incident to emptying affects the subsequent activity for a considerable period of time. Therefore repeated absorption periods of various total durations have been used, and the final volume measured by the reference constituent method, eliminating the errors due to incomplete emptying.

METHODS AND RESULTS. Chronic Thiry-Vella ileal loops, 40 cm. in original length, were prepared in dogs as described by Dennis (1939) except that the ends of the loops were tunnelled subcutaneously over the costal margin to facilitate closure by simple compression during observation periods. Out of a larger number studied, two animals showing the closest constancy of absorption rates under controlled conditions in repeated observations were selected for the main studies. All dogs were carefully trained to lie quietly on the observation table without restraint during periods of study. Prior to each experiment the loop was washed with 50 cc. 0.92 per cent NaCl solution at 38°C. introduced by means of a no. 14 French soft rubber catheter provided with multiple holes near the tip and inserted to the midportion of the loop. This fluid was removed with a gentle stream of air and 15 minutes allowed to elapse leaving the air-filled catheter in place before the absorption experiment was begun. All fluids were introduced and removed through the catheter which was emptied of fluid by introduction of 2 cc. of air during absorption periods.

Volumes of fluid in the loop were determined by the *reference constituent* method, employing either sulfate or chloride for that purpose. When sulfate-containing solutions were studied that amount of the fluid remaining in the gut at the end of a period of absorption which was readily withdrawn in a syringe connected to the catheter was so removed. Immediately thereafter a known volume of 0.92 per cent NaCl was flushed through the loop and collected. Both fluids were analyzed for sulfate and the true final volume calculated as the sum of the final fluid withdrawn by syringe plus the quantity,—wash volume times its sulfate concentration divided by the sulfate concentration in the final fluid. In using chloride as the reference constituent the procedure is the same in principle except that isotonic sucrose is used as the wash solution and calculations are made from chloride concentrations. By these methods volumes of fluid in loops can be measured with an accuracy of $\pm$ 0.4 cc. Water cannot be used as a wash fluid because, as Dennis (1939b) has shown, the ability of the intestine to absorb against gradients is impaired or temporarily abolished by short periods of exposure to pure water. Chloride was determined by the Van Slyke method and sulfate by the titrimetric benzidine procedure.

Experiments of two sorts have been performed and examples will be presented in this paper. In one case absorption over a given period of time (12 min.) was studied repeatedly on the same loop, using varying

proportions of two salts, NaCl and Na_2SO_4 in isotonic solutions in successive experiments at regular intervals. The period between trials definitely influences the results, absorption being slower when the interval is short.

TABLE 1

EXPERIMENT NUMBER	ORIGINAL CONCENTRATION		PERIOD OF ABSORPTION	FINAL CONCENTRATION		CALCULATED OSMOTIC EQUIVALENT OF NaCl AND Na_2SO_4 IN SOLUTIONS AS M. EQUIV. NaCl*	
	Cl	SO_4		Cl	SO_4	Initial	Final
	m. equiv.	*m. equiv.*	*minutes*	*m. equiv.*	*m. equiv.*		
a 1	156.0	0	10	112.0	0	156.0	112.0
2	141.3	25.2	12	74.5	58.0	167.2	111.1
3	131.0	42.0	12	59.0	98.0	157.5	120.8
4	79.0	126.0	12	11.0	203.4	158.4	139.1
5	43.6	202.0	12	1.6	254.0	170.9	161.7
b 1	156.0	0	12	125.0	0	156.0	125.0
2	141.3	25.2	12	109.5	26.8	167.2	116.4
3	79.0	126.0	12	33.7	160.0	158.4	134.5
4	43.6	202.0	12	16.3	218.0	170.9	153.6

* Calculated as the sum of chloride concentration plus 0.63 times that of sulfate in milliequivalents.

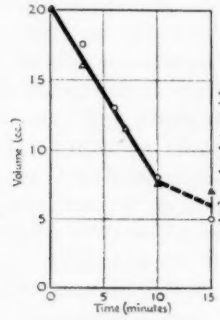

Fig. 1a

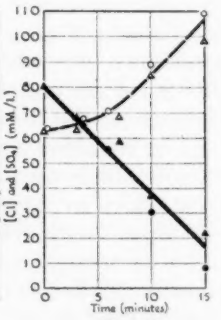

Fig. 1b

Fig. 1a. The rate of volume decrease during absorption of an isotonic solution containing originally 63 mM Na_2SO_4 and 78 mM NaCl from a chronic Thiry-Vella ileal loop in the dog. For further details see text.

Fig. 1b. The changes in concentrations of $\bar{Cl}$ and $\bar{SO}_4$ over time in the experiments shown in figure 1a.

The composition of the fluids introduced in the experiments reported in figure 2 a and b is shown in columns 2 and 3 of table 1. In determining the rate of water absorption over shorter intervals of time the method used was in general the same except that the duration of absorption, rather than

the composition of the fluids was varied. Twenty cubic centimeters of solution containing 78 mM NaCl and 63 mM Na_2SO_4 were introduced into the loop and after a certain time interval withdrawn as described above.

The composite results of experiments upon the influence of the relative concentrations of sulfate and chloride in originally approximately isotonic mixtures of their sodium salts on the absorption process are shown in figure 1 a and b. The several rates measured are plotted against observed mean concentrations of sulfate and chloride during the period of absorption. The mean concentration over the time of absorption has been selected for comparison because original and final concentrations differ substantially from one another. The general significance of the results would not alter, however, if original concentrations were used for reference.

In figures 2a and b one sees that the absolute amount of chloride absorbed falls off as its mean concentration in the solution diminishes, but that the proportion of the total present which is absorbed increases with increasing sulfate concentration. This is evident from the course of the chloride clearance rate[1] curve. This calculation has no necessary connection with any particular mechanism of absorption. It is employed here simply as a measure of the rate of removal of chloride, in terms of cubic centimeters of fluid, *the chloride in which is removed from the gut, per unit of time.* It can be seen that the chloride clearance rate more than doubles on increasing the sulfate from 0 to 240 m. equivalents per liter.

Coincident with the decrease in absolute absorption of chloride with the higher concentrations of sulfate in the gut fluid, shown in the upper segments of figures 2 and 3, is an increase in absorbed sulfate. Our experiments on sulfate clearance are too few to allow a statement as to whether the relative absorption rate alters with concentration. This problem is much complicated by the fact that the degree of impermeability of the intestinal epithelium to sulfate is variable in different animals and under different conditions in the same animal. Poisoning or injury of other sorts makes the gut wall more permeable to sulfate.

The net rate of fluid absorption is also a function of the relative proportions of sulfate and chloride. However, over the range between 40 and 220 m. equiv. of sulfate there is by no means a direct proportionality in fluid moved. For a five-fold increase in sulfate concentration there is less than a 50 per cent decrease in net water movement, and over a considerable range of sulfate values there is no appreciable change in the fluid absorption rate. This is in spite of 100 per cent changes in chloride clearance over the same sulfate range. Thus although there was no more

[1] R_0 is the clearance rate for chloride. Its calculation is from the equation $\frac{CV}{C_0V_0} = \frac{V}{V_0}^{\frac{R_0}{D}}$ (Peters and Visscher, 1938).

net fluid transport, the fluid in the gut was nevertheless being cleared of chloride faster at sulfate values of 160 than at 40 m. equiv. This observation is extremely hard to harmonize with any theory of uni-univalent

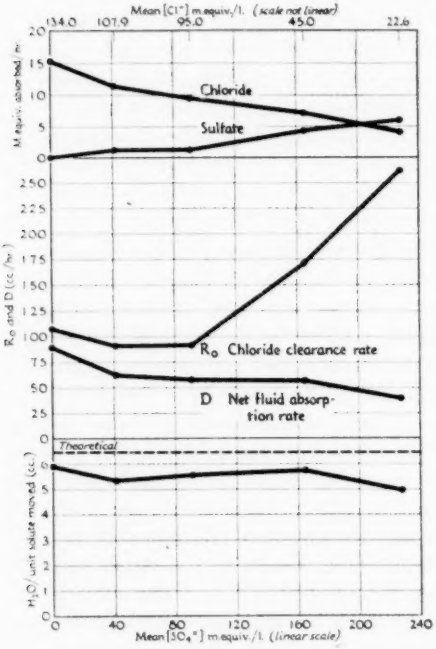

Fig. 2a

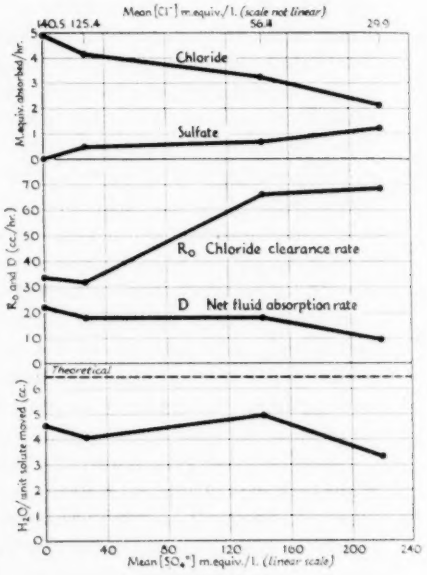

Fig. 2b

Fig. 2a. The rates of chloride and sulfate absorption (upper graph), clearance of intestinal fluid of chloride, R_0, adsorption of water, D, and solvent per unit of solute removed, from a chronic Thirty-Vella ileal loop in a one-year-old dog in relation to average concentration of NaCl and Na_2SO_4 in initially isotonic solutions placed in the loop. The values given on the abscissae are arithmetic mean concentrations determined from observed concentrations of Cl and SO_4 at the beginning and end of a 6 minute absorption period. Each set of vertical points represents a separate experiment on the same loop. In each experiment, except at zero concentration of SO_4 where a shorter period was used, absorption was studied over twelve minutes with samples drawn at six minutes. The volume change, D, was calculated for the twelve minute period, other values are for the second six minute period.

Fig. 2b. The same in the case of an ileal loop in a 6-year-old dog. Note the slower rates at all relative concentrations.

salt absorption which would involve one-way fluid movement related in any way to the salt movement. A fluid circuit theory is consistent with the facts.

The data available in these experiments allow one to calculate the

amount of water moved per osmotic equivalent of salt absorbed. This calculation acquires importance in view of the fact previously mentioned that these solutions become significantly hypotonic during absorption, as measured by vapor tension methods. Dog's blood is isosmotic with 156–162 mM NaCl. At the total ionic concentrations in question 1 mM Na_2SO_4 is the osmotic equivalent of 1.26 mM NaCl, according to vapor tension measurement. From these data one can calculate the osmotic equivalent of sulfate and chloride in terms of equivalent quantities of chloride alone. By dividing the figure for net water moved per unit of time by the osmotic equivalent of salt as NaCl moved, one obtains the observed value for water moved per osmotic equivalent of the two salts moved. The interesting result has come out of these calculations, shown in the lowest curves in figures 2a and b, that in no instance has the salt carried its isotonic equivalent of water with it from gut to blood, in terms of net water movement. The interpretation of this perfectly regular observation is not entirely obvious. Some solute undoubtedly enters the gut from the blood under the conditions in question (Peters and Visscher, 1938). However, as noted above, the gut fluid is hypotonic and therefore normal osmotic forces would be tending to move water from gut to blood. The main salt movement is of NaCl against its concentration gradient. It seems remarkable that this latter process outdistances the water movement. The fact that some solutes, such as urea and bicarbonate, may be entering the gut, the former in small and the latter in significant amounts (Lifson, 1940), does not clarify the question on this score because still one is confronted with the fact that during the process of absorption the gut fluid is significantly hypotonic in spite of the entrance of those substances. The simplest alternative or supplementary assumption which would account for the facts as observed would appear to be the hypothesis that a hypotonic solution entered the gut simultaneously with the exit of fluid.

Reference to table 1, columns 7 and 8, will show the further interesting fact that the calculated osmotic equivalent of the total NaCl and Na_2SO_4 in solutions in the gut after 10 to 12 minutes of absorption deviates from the isotonic equivalent of 159 mM NaCl to a greater extent the less Na_2SO_4 is present. These several facts are in apparent contradiction to the view that isotonic NaCl is absorbed by simple transport in isotonic solution.

CONCLUSIONS

1. A method is described for the measurement of water and solute absorption from intestinal loops, employing a reference substance.

2. Volume decrease during absorption is approximately linear, within the limits of accuracy of measurement, during the first 10 minutes.

3. The chloride clearance rate is a direct but non-linear function of the concentration of sulfate in originally isotonic solutions containing sodium salts of the two anions undergoing absorption.

4. The absolute absorption rates of both sulfate and chlorides in isotonic mixtures of their sodium salts are direct functions of their individual concentrations.

5. The net fluid absorption rate from isotonic solutions of varying proportions of NaCl and Na_2SO_4 is inversely related to the concentration of sulfate but not linearly so.

6. The net solute absorption is not equal to the calculated osmotic equivalent of water of the salt moved. The net effect is therefore an absorption of a hypertonic solution. As to mechanism the more probable reality is a two way movement in which a hypotonic solution enters the gut while salt solution leaves it to enter the blood. Intestinal transport may be looked upon as the algebraic sum of two-directional movements.

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THE SENSITIZATION OF THE DENERVATED HEART TO ADRENALINE

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Shortly after denervation certain structures of the body have been shown to become sensitized to chemical agents, natural or otherwise. Evidence for this "law of denervation" has been recently reviewed by Cannon and Rosenblueth (1937) and by Cannon (1939). For example, smooth and skeletal muscle, after being denervated, become more responsive than before to chemical stimulation. Heart muscle deprived of its nerves might be expected to respond in like manner. Sawyer, Hampel and Ring (1938), however, before using the denervated heart as an indicator in chronic experiments, tested it for sensitivity to adrenaline and drew the conclusion that it was not sensitized by denervation. The present paper deals with a further study of the response of the denervated heart to adrenaline.

METHOD. Healthy young cats were selected for each experiment. Under ether anesthesia the heart was surgically denervated according to the technique described by Cannon, Lewis and Britton (1926). The animals were then placed in warm cages for about 24 hours before being subjected to the first experiment. About 3 days after operation they began to eat, and within a week they were taking their food quite normally. They were allowed to convalesce 7 days before a second experiment was performed. Seven-day intervals separated subsequent experiments. Each animal showed a moderate loss of weight after the operation, with a gradual return to normal in about a month. Experimental results were not affected by weight variations.

For the experimental observations nembutal (0.7 cc. per kgm.), injected intraperitoneally as recommended by Hampel (1935), produced the proper depth and evenness of anesthesia during a period of 3 hours or more. At the conclusion of an experiment 60 cc. of warm normal salt solution was injected intraperitoneally, a treatment which seemed to have beneficial effects.

The anesthetized animal was placed on its side on an animal board, and the hind limbs were tied down in such manner that the femoral vein was readily accessible for exposure. The area over the vein was shaved and

cleansed with alcohol. A 25-gauge hypodermic needle, which had been soldered at right angles to the long axis of a copper rod, was inserted into the vein and held in place by a clamp. A continuous slow saline drip was then connected to the needle. It could be readily detached and temporarily replaced by a 1-cc. syringe.

The apparatus for recording the heart beat (Cannon, Lewis and Britton, 1926) consisted of a tambour which was placed between the animal board and the cat's chest. It was adjusted to the area of maximal cardiac impulse by packing cotton beneath it. The tambour was connected by rubber tubing to a sensitive Marey capsule the lever of which recorded the heart beats on a kymograph. A glass T-tube inserted into the rubber tubing made possible regulation of the pressure within the tambours and amplification of even poorly transmitted heart beats without compression of the chest wall. This fact is stressed because of marked changes in heart rate which may result from asphyxia. The interval of 24 hours between the operation and the first experiment was intended to avoid this possible complication, which sometimes resulted from post-operative pneumothorax.

At the beginning of each experiment a 10-γ solution of adrenaline was prepared from a 1:1,000 stock solution (Parke, Davis and Co.), with normal saline as a diluent. One cubic centimeter, containing from 0.05 γ to 10.0 γ of adrenaline, was injected into the femoral vein at an even rate in 10 seconds. Adrenaline deteriorization was carefully avoided. Solutions from 0.5 to 2 γ per cc. lost much or all of their effectiveness after standing for 20 minutes; in solutions containing 0.1 γ per cc. a similar loss occurred in half that time. Therefore, adrenaline solutions between 10 γ and 3.3 γ per cc. were not permitted to stand longer than 20 minutes; when the concentration was between 2 and 0.5 γ per cc. the drug was injected within 10 minutes after its dilution; and for doses of 0.25 γ or less not more than 5 minutes intervened before injection.

Twenty-five seconds before adrenaline was injected, a graphic record of the heart beat was started and was run for about 80 seconds. The heart rate for 10 seconds previous to the injection and the maximal rate during the response were counted.

The animals rested upon an electric heating pad throughout the test. Rectal temperature varied not more than 1°C. in any experiment. Within this range there appeared to be no correlation between temperature and heart-rate changes.

Results. Doses of 0.05, 0.1, 0.25, 0.5, 1.0, 2.0, 3.33, 5.0 and 10.0 γ of adrenaline were used. The results occasionally obtained with the minimal dose (0.05 γ) may not be significant, since the increase over the basal rate was small and fell within the range of error of the methods employed. When amounts of adrenaline greater than 2 γ were administered, the denervated heart response in the same and in different animals varied

considerably, i.e., the percentile increase in the basal rate between the 8th and the 29th day as compared with that on the 1st day was either increased, unchanged or decreased. Moreover, in 3 animals cardiac arrhythmias developed in response to doses greater than 2 γ as early as 15 days after denervation.

Since results obtained with very small and with large doses of adrenaline were variable and unreliable, the data obtained with moderate doses

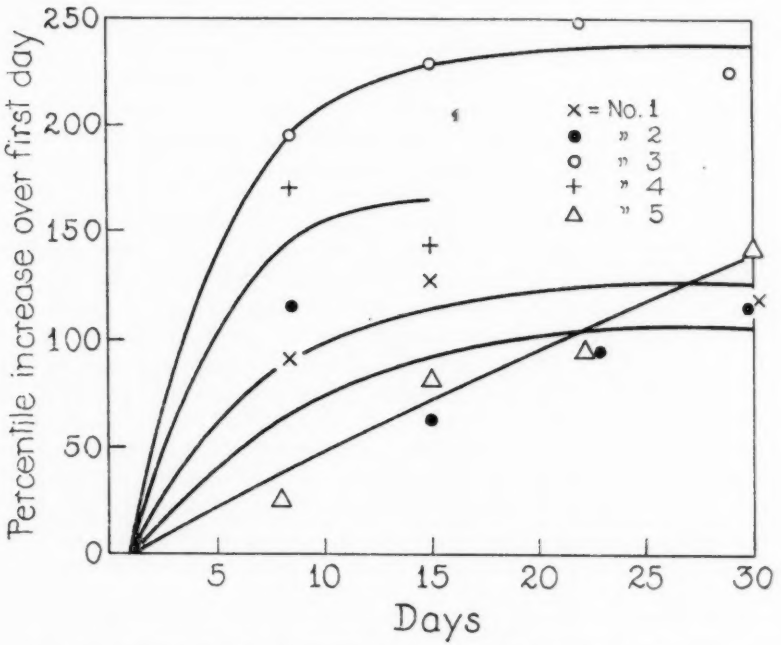

Fig. 1. Response of the denervated heart to 0.5 γ of adrenaline in 5 cats. The ordinates represent percentile increase in basal rate over that on the 1st day; the abscissae represent days after denervation.

(ranging between 0.1 and 2 γ) are presented as evidence for sensitization of the heart after being denervated.

For each dose from 0.1 up to and including 2 γ, the cardiac reactions to adrenaline fell into a general pattern. The percentile increase over the basal rate for a given dose rose rapidly between the 1st and 8th day after the denervation. After the 8th day the percentile increase over that of the 1st day either increased slowly or leveled off (fig. 1). One cat responded to 0.25 and 0.5 γ in an unusual manner in that the increases over the 1st day rose steadily for more than 3 weeks (cat 5, fig. 1). The responses to the other doses, however, were typical in this animal.

In table 1 the results for each dose of adrenaline are summarized in a representative animal. The percentile increases in heart-rate response over that on the 1st day are shown to vary from a minimum of 3 per cent on the 29th day with 2 γ to a maximum of 390 per cent on the 29th day with 0.1 γ (cat 2, table 1). It is also evident that the small doses of adrenaline give the greatest percentile increase over that on the 1st day. For example, on the 8th day the percentile increase for the 5 cats varied between 129 and 266 per cent with 0.1 γ and between 14 and 124 per cent with 2 γ. These results indicate that smaller doses of adrenaline, near the minimal, demonstrate most clearly the sensitizing effect of denervation.

TABLE 1

Cat 2. Response of denervated heart to adrenaline

ADRENALINE DOSAGE γ	DAYS AFTER DENERVATION OF HEART															
	1			8				15				22				29			
	Heart rate per minute		Per cent increase over basal rate	Heart rate per minute		Per cent increase over basal rate	Per cent increase over 1st day	Heart rate per minute		Per cent increase over basal rate	Per cent increase over 1st day	Heart rate per minute		Per cent increase over basal rate	Per cent increase over 1st day	Heart rate per minute		Per cent increase over basal rate	Per cent increase over 1st day
	Basal	Maximum after adrenaline		Basal	Maximum after adrenaline			Basal	Maximum after adrenaline			Basal	Maximum after adrenaline			Basal	Maximum after adrenaline		
0.1	119	121	1.7	135	142	5.2	203	148	157	6.1	258	152	164	7.9	365	133	144	8.3	390
0.25	127	135	6.3	146	163	11.7	85	169	185	9.5	51	165	191	15.7	149	158	180	14.0	122
0.5	124	140	12.9	144	184	27.8	116	168	204	21.4	63	167	209	25.2	95	151	193	27.8	115
1.0	118	144	22.0	142	203	43.0	95	152	208	37.0	68	163	237	45.5	107	137	196	43.0	95
2.0	129	178	38.0	144	228	58.2	53	173	243	40.5	8	164	248	54.3	43	164	228	39.1	3

After the 15th day of denervation, with doses of adrenaline over 1 γ, the heart in different animals exhibits either a decline or a rise in percentile increase in rate over that on the 1st day. In order to trace the development of sensitization to its completion, therefore, smaller doses than 1 γ must be used. In the present experiments the development of sensitization was followed for 29 days. The data do not indicate that the process even then had reached its conclusion.

The increase over the basal heart rate at successive 15-second intervals after the administration of 1 γ of adrenaline was analyzed on the 1st and on subsequent days after denervation. The percentile increase over the basal rate in one minute showed definite elevation from the 1st to the 15th day. Additional evidence of sensitization was also revealed as shown by a prolongation of the increase in rate on the 8th and on subsequent days as compared with that on the 1st day. For example, in cat 2 the heart rate

on the 1st day after denervation increased 4, 6, 4 and 3 beats at successive 15-second intervals after the injection of adrenaline; whereas on the 15th day it increased 4, 13, 12 and 10 beats, thus failing remarkably to decline to the basal rate at the end of 1 minute. Obviously adrenaline has a longer action on the sensitized heart. Moreover, the percentile increase over the basal rate is greater as sensitization develops if it is computed over 1-minute intervals (86 per cent in cat 2, on the 15th day, with 1 γ) instead of being computed on the basis of the maximal 10-second increase in rate (68 per cent). This is likewise due to a prolongation of adrenaline action on the denervated heart.

A manifestation of denervation other than heart-rate changes was noted and seems worthy of mention. The time interval between the injection of adrenaline and the maximal 10-second increase in heart rate was in every instance shorter on the 1st than on the succeeding days after the denervation. For example, in cat 4, with the injection of 1 γ of adrenaline, the maximal increase in rate occurred between 7 and 17 seconds on the 1st day, between 15 and 25 seconds on the 8th day, and between 17 and 27 seconds on the 15th day. There was, however, no close correlation between the length of the interval before the maximal increase of rate occurred and the duration of denervation or the dosage of adrenaline. The reason for the occurrence of this phenomenon is obscure.

REMARKS. The results above reported disclose that within limits as time passes after denervation there is an increased heart rate in response to a given dose of adrenaline. The heart deprived of its nerve supply may therefore be considered to be sensitized to adrenaline. Justification for this assumption has been discussed by a number of authors (see Hampel, 1935). An explanation of the sensitizing of denervated structures is not revealed in the present experiments. The most plausible theory remains to be confirmed, i.e., that adrenaline and other substances act with more effectiveness because of an increase in permeability of cell membranes (Rosenblueth and Morison, 1934).

As previously noted, Sawyer, Hampel and Ring (1938) drew the conclusion that the heart is not sensitized to adrenaline by denervation. They used a method which differed from the present one in several respects: the cats were not anesthetized when adrenaline was injected, the adrenals were inactivated and the heart rate was not continuously recorded but was counted with a stethoscope. Nevertheless, their table, summarizing the results of a representative animal, shows upon careful analysis that, contrary to their inference, they actually demonstrated that denervation sensitizes to adrenaline. They injected 1 γ of adrenaline into the femoral vein 1, 2, 3, 4, 5, 9 and 12 days after denervation and counted the heart rate at successive 15-second intervals for 1 minute. If the increase in rate for 1 minute is counted on the 1st and the 12th days after denervation,

the percentile increase over the basal on the 12th day is found to be 60 per cent greater than that on the 1st day. An analysis of the present experiments using the same dose of adrenaline and identical methods of calculation not infrequently reveals a much higher percentile increase. In cat 2, the basal rate for 60 seconds increased to 86 per cent by the 15th day. Except two animals which showed increases of 52 and 56 per cent on the 15th and 8th day respectively, the percentile increase over the basal rate in 45 seconds ranged from 100 to 300 per cent from the 8th day of denervation onwards. That the percentile figures derived from the present experiments are higher than those obtained from the results reported by Sawyer, Hampel and Ring (1938) may be due to the differences in experimental procedures mentioned above. There is no doubt, however, that sensitization to adrenaline by denervation is revealed in both instances.

From the findings reported above, it is apparent that if the chronically denervated heart is to be used as a reliable indicator in experiments in which results are based upon variations in the response of the heart rate to adrenaline, the dosage and time after denervation must be carefully controlled.

SUMMARY

1. The denervated heart of 5 cats was tested for sensitization to adrenaline. The heart-rate response to graded doses of adrenaline (0.05 to 10.0 γ) was observed 1 day after denervation and every 7 days thereafter for 29 days.

2. Sensitization could not be reliably demonstrated with small (0.05 γ) and large (3.33 to 10.0 γ) doses of adrenaline. The larger doses frequently induced arrhythmias by the 15th day.

3. Definite sensitization always developed within 8 days after denervation (fig. 1).

4. A slight and progressive increase in sensitization usually occurred, with small doses of adrenaline, from the 8th to the 29th day following denervation, and there was no indication that sensitization was complete at 29 days (table 1).

5. After the 15th day of denervation it was necessary to use doses of less than 1 γ to detect further development of sensitization; doses near the minimal effective dose revealed it more clearly.

6. The response of the denervated heart to adrenaline was prolonged as denervation developed.

7. The latency of the maximal adrenaline effect on the 8th and subsequent days was increased over that on the 1st day.

8. The response of the denervated heart to adrenaline should not be used as an indicator in chronic experiments without first recognizing the existence of sensitization due to the denervation.

I wish to express my thanks to Dr. W. B. Cannon for suggesting this problem, and to Dr. R. Hodes and Dr. A. Ravin for their assistance and helpful advice.

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THE RELATION OF CONTRACTION OF DIFFERENT REGIONS OF THE VENTRICLE OF THE TURTLE TO THE RISE OF INTRAVENTRICULAR PRESSURE[1]

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In experiments recently reported from this laboratory (1) concerning the relation between electrical and mechanical events in the turtle's heart, local contractions of the surface musculature of the ventricle were recorded by means of a myograph simultaneously with potential time curves from the same region. The myograph, which operates by changing the resistance to an electrical current flowing through it, was described in detail in this communication. Since our report, the work of Shannon and Wiggers (2) on the isometric period of the ventricle of the turtle and frog has appeared. These investigators establish the presence of an isometric period in the sense of a rise of intraventricular pressure preceding by a definite interval the rise of aortic pressure. This finding led them to question the validity of myographic curves as indicating the onset of local muscle shortening. The work reported in the present communication is a further examination of this question by a study of the relations existing between myograms and pressure curves.

METHODS. For recording pressures a Gregg type of optical manometer, similar to that employed by Shannon and Wiggers was used.[2] To provide for greater convenience and flexibility, recording was made by the use of a photoelectric system instead of by the usual optical method. A light source, the manometer and a photoelectric cell were mounted together on a rigid metal frame which could be adjusted with respect to the heart so that only a short (10 cm.) lead tube was necessary for connecting the hypodermic needle (size 18) with the manometer. Heavy, tightly stretched rubber from an inner tube was used for membranes. The changes in current flowing through the photoelectric cell, due to changes in the amount of incident light reflected by the membrane mirror, were amplified by means of a direct current amplifier (3) and recorded by a cathode ray oscilloscope on bromide paper. For recording pressures from the ventricle and great

[1] Supported in part by a grant from the Wisconsin Research Foundation.

[2] We wish to acknowledge our indebtedness to Doctor Wiggers for the loan of a manometer for duplication and for invaluable criticism and advice in this work.

vessels, the light and electrical amplification were adjusted so that the movement of the cathode beam across the tube surface (a distance of 3 in.), corresponded to a change of pressure of about 30 mm./Hg. In a few experiments intra-auricular pressure was recorded simultaneously with myograms from the surface of the right auricle. The same membrane and manometer were used and the necessary sensitivity obtained by increase in the electrical amplification. Local shortening of the surface musculature from various regions on the ventricle were recorded by the myograph and a second direct current amplifier and oscilloscope simultaneously with intraventricular pressure. Mensuration of the curves was done with a micrometer comparator with 40x magnification (4).

Most of the experiments were done on large specimens of the snapping turtle (Chelydra serpentina). The large ventricle of this species made possible the recording of myograms from as many as 18 separate regions on the anterior surface. In several experiments the smaller turtle Chrysimus was used, and one experiment was performed on a large soft shell turtle (Amyda spinifer). In most cases artificial respiration was employed.

RESULTS. The intraventricular pressure curve of the turtle shows an initial slow rise of pressure, amounting to several millimeters of mercury and lasting from 0.10 to 0.12 sec. In the measurement of these curves in the magnifying comparator, the horizontal hair of the microscope is adjusted to coincide with one edge of the curve and the carriage bearing the microscope moves with the micrometer screw until the edge of the curve leaves the hair. By this procedure, the onset of the initial slow rise of pressure may be determined quite accurately. The end of this period is indicated by a sudden increase of gradient, the pressure rising rapidly toward its maximum.

Myograms, registering the shortening of local regions of the ventricle, and recorded simultaneously with intraventricular pressure, show that at all surface regions, the onset of shortening occurs within the period of initial slow rise of pressure or follows the end of this period by a brief interval. Using the start of the rise of pressure as a reference for the myograms, the first region of the anterior surface of the ventricle to shorten is found to be the left base, and the onset of shortening in this region is coincident with the first detectable rise of intraventricular pressure. The apex and right base, which are the last regions to shorten, start to shorten late in the initial period of intraventricular pressure rise, or follow the end of this period by 0.01 to 0.02 sec. In other regions, the onset of shortening occurs at various times during the initial period of rise of pressure. The snapping turtle and Chrysimus showed the same sequence of involvement in the shortening process as was found in our previous work, in which a peak of a constant unipolar curve was used as a reference for the onset of shortening as recorded from various local regions by the myograph.

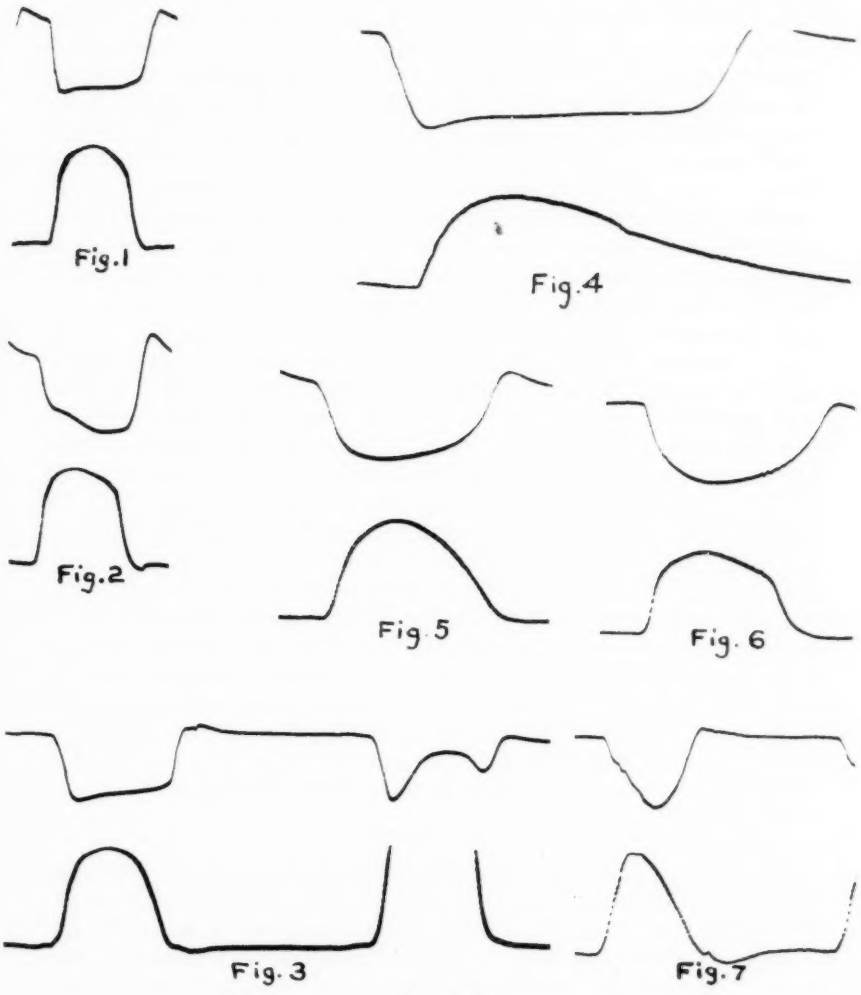
Figures 1 and 2 show the type of curves obtained from the ventricle. The upper curve in each record is the myogram, shortening of a local region of the ventricular surface being indicated by a downward movement. The lower curve records intraventricular pressure, a rise being indicated by an upstroke. In figure 1, the myograph was on the left ventricular base of a snapping turtle. The onset of shortening is coincident with the first detectable rise of intraventricular pressure. The change to an increased gradient in the pressure curve occurs 0.088 sec. later. The maximum intraventricular pressure is 26 mm./Hg, the heart rate 23 per min. Figure 2 is from the same heart and records the shortening of a region on the anterior midsurface of the ventricle. The onset of shortening follows the initial rise of intraventricular pressure by 0.027 sec., and precedes the onset of the sharp rise by 0.063 sec. The heart rate is 23 per min., the maximum pressure 25 mm./Hg. The speed of recording was 10 mm. per second in these two figures.

That local shortening of ventricular muscle occurs even though a change of volume of the chamber is absent, is illustrated in figure 3. Between the two cycles shown the great vessels were clamped. The result is a modification of the myogram in a manner indicating that full shortening no longer occurs, but the onset of shortening has the same time relation to the rise of intraventricular pressure as it did previous to the occlusion of the great vessels. Before clamping, the maximum intraventricular pressure was 25 mm./Hg. Clamping caused a marked increase. The record was made at a speed of 21.5 mm. per sec.

When intrapulmonic or intra-aortic pressure is recorded instead of intraventricular pressure, the onset of shortening of a local region of the ventricle precedes the rise of pressure by a longer interval as an expression of the isometric period. Figure 4 is an example. The upper curve records the shortening of a region at the left base of the ventricle of a snapping turtle. The lower curve is a record of intra-aortic pressure. The interval between the start of the descent of the myogram and the start of the rise of pressure is 0.29 sec. The record was made at a speed of 21.5 mm. per sec.

The results obtained from the two other species of turtles used were the same as those from the snapping turtle. Figures 5 and 6 are records, similar to those shown in figures 1 and 2, but obtained from the ventricles of Chrysimus and Amyda respectively. In figure 5, the myograph was on the left base. The onset of shortening is coincident with the initial rise of intraventricular pressure. The maximum pressure is 25 mm./Hg. In figure 6, the myograph was to the left of the mid ventricle. The onset of shortening follows the initial rise of pressure by 0.018 sec., and precedes the sharp rise by 0.063 sec. The maximum pressure is 30 mm./Hg. In both of these records the speed of recording was 21.5 mm. per sec.

The relation of local shortening of a region of the auricle to the rise of intra-auricular pressure is illustrated in figure 7. The myograph was on the middle of the anterior surface of the right auricle of a large snapping



Figs. 1–7

turtle. The onset of shortening follows the start of the rise of pressure by 0.05 sec. The total change of pressure is a little more than one millimeter of mercury. Speed of recording, 21.5 mm. per sec.

Discussion. If it is assumed that the volume of blood in the turtle's ventricle is constant during the isometric period, the question arises as to how the shortening of the muscle can occur during this period. It is conceivable that with constant volume, local shortening might be made possible by local lengthening of the muscle in other regions. Against this interpretation is the fact that we have found no region in the normally beating ventricle in which the myogram shows any evidence of initial dilatation. It would appear that an adequate explanation of the shortening during the isometric period is present in the obvious change in shape that the ventricle undergoes during this period. The relaxed ventricle is approximately oval in shape. On contraction, the lateral walls are drawn in, the ventral-dorsal diameter increased, and the ventricle approaches the form of a sphere. This change in shape occurs early in the isometric period, and is probably in large part completed by the end of the initial slow rise of intraventricular pressure. This period has a duration of about 0.12 sec., and is associated with a rise of pressure of only a few millimeters of mercury. With the end of the initial period and the entrance of all or nearly all of the muscle in the shortening process, the intraventricular pressure rises rapidly to exceed the pressures in the great vessels and to end the isometric period. The earliest phase of systole of the ventricle is thus characterized by a marked change in shape of the chamber with little rise of pressure of the blood contained within it. Since the sphere has the smallest surface for a given volume, shortening of the muscle occurs without reduction of the volume of the blood in the ventricle.

That different parts of the dog's auricle enter into contraction at different times, has been shown by comparison of myograph and intra-auricular pressure curves (5) and by determination of the time relations between local shortening and electrical events (6). It has also been concluded, in large part from indirect evidence, that the same applies to the dog's ventricle (7). Wiggers introduced the term "fractionate contraction" to indicate the shortening of local muscle units (5). From the present report it is clear that this situation exists in the ventricle of the turtle.

CONCLUSIONS

The rise of pressure in the ventricle of the turtle during systole is characterized by an initial slow rise, amounting to a few millimeters of mercury and lasting about 0.12 of a second. This is followed by an abrupt increase of the pressure gradient. Local shortening of various regions on the anterior surface of the ventricle begin during the initial period of pressure rise or within an interval of several hundredths of a second after its termination. Shortening in all regions thus occurs before the pressure has risen sufficiently to force blood into the great vessels and reduce the volume of the ventricle. This shortening is made possible by change in

shape of the ventricle early in its contraction to approximate that of a sphere. Myograms from the surface of the ventricle of the turtle hence afford an adequate criterion for the onset of the local shortening process and for a comparison of electrical and mechanical events.

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ACETYLCHOLINE-EQUIVALENT CONTENT OF THE NASAL MUCOSA IN RABBITS AND CATS, BEFORE AND AFTER ADMINISTRATION OF ESTROGEN[1]

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There is both clinical and experimental evidence to show that the condition of the nasal mucosa is affected by sex hormones, particularly estrogen. The literature on this topic is both old and extensive (cf. Reynolds, 1939a; Hamblen, 1939; Fluhmann, 1939). In general, it may be said that when there is an abundance of estrogen in the blood, the nasal mucosa may be moderately hyperemic. Recently, Bernheimer and Soskin (1939) observed that while estrogen brings relief in some cases of atrophic rhinitis, it does not invariably do so; such patients, some of whom were women with normal menstrual (i.e., hormonic) cycles, were relieved, however, by topical application of prostigmine. These facts, along with other data which show that estrogen exerts within one hour a cholinergic action on the uterus of the rabbit (Reynolds, 1939b; Reynolds and Foster, 1939) suggest that estrogen may bring about in the nasal mucosa an increase in the concentration of free acetylcholine. This may result from increased production of the substance, or decreased destruction through diminished activity of cholinesterase.

In accordance with the foregoing considerations, the following experiments were carried out. These were planned to demonstrate the extent to which there may be a change in the concentration of an acetylcholine-like substance in the nasal mucosa following injection of estrogen. The method of tissue extraction and testing is that used heretofore by us (Reynolds, 1939b; Reynolds and Foster, 1939), and is that recommended by Chang and Gaddum (1933). The method is sensitive to 0.01–0.05 gamma of acetylcholine. Tissues from ovariectomized rabbits and cats were used. Castration was performed from six to seventy days prior to an experiment, although eighteen to twenty-one days was most often the elapsed time between operation and experiment. Cats were used in addition to rabbits since estrogen has no measurable effect on the concentration of acetylcholine in the uterus, as in the rabbit (Reynolds and Foster, 1940). The probable significance of this difference is discussed in the earlier paper.

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The experiments were carried out in the following way. On the day of an experiment, two rabbits were anesthetized by intramuscular injection of Dial (Ciba). One was used as an untreated control, the other received a subcutaneous injection of estrogen (Amniotin, Squibb; Progynon-B, Schering; Ben-Ovocylin, Ciba). Each of these estrogens exerts a cholinergic action on the uterus of the rabbit within an hour (Reynolds and Foster, 1939). The amounts used were 400 to 10,000 units of Amniotin, and from 0.05 to 2.0 mgm. of the estradiol compounds. When the animals were fully anesthetized, the skin over the nasal, frontal, and maxillary bones was removed. The nasal septum was cut across with a sharp scalpel and the nasal bones loosened laterally by means of bone forceps. They were then torn loose from their attachments by pulling dorsally with a hemostat on the nasal septum. In this way, the turbinate and ethmoid bones were exposed. The mucosa was then stripped off in as large pieces as possible. These were weighed immediately on a glass slide and immersed in 10 per cent tri-chloracetic acid. Extraction for an acetylcholine-like substance was then carried out. If an animal suffered from any obvious nasal congestion or infection, the tissue was not used. The quantities of tissue (mucosa plus some cartilage and superior turbinate bones) ranged from 0.5 to 1.5 grams per animal, although most often the quantity of tissue was about one gram. In every case, however, the amount of extract available was so small that it was impossible to employ the usual confirmatory tests for acetylcholine. Consequently, in the account which follows, we refer to the acetylcholine-equivalent content of the nasal mucosa, rather than to the acetylcholine content of the tissue.

Results. *Activity of extracts from untreated animals.* In both cats and rabbits, the acetylcholine-equivalent content of the nasal mucosa is nil, or inappreciable in the majority of animals. This is shown by the following considerations.

It was observed that tissues from 59.1 per cent (13 of twenty-two) of the untreated rabbits and from 80 per cent (eight out of ten) untreated cats contained no measurable amount of an acetylcholine-like substance. Of the remaining 40.9 per cent of rabbits and 20 per cent of cats whose tissues yielded active extracts, the quantities were small; this is shown in figure 1, where the average concentration of an acetylcholine-like substance for each group is represented by the shaded bars, individual determinations from the several experiments, by dots. It will be seen that both the mean values and the range of individual points indicate a low concentration of an acetylcholine-like substance in the preponderance of cases.

Activity of extracts from estrogen-injected rabbits and cats. In contrast to the results of the foregoing group of experiments, the data from this group show that one hour after injection of estrogen, there is an appreciable

increase in concentration of an acetylcholine-like substance in the nasal mucosa. This was observed in both rabbits and cats.

The number of rabbits yielding measurably active extracts was increased to 75 per cent (eighteen out of twenty-four), the number of cats, from 20 per cent to about 90 per cent (ten of eleven cats). The mean concentrations and the data from individual experiments are shown in figure 1. In rabbits, the mean concentration was increased from 0.028 to 0.093 gamma per gram of tissue, or about a three and half-fold increase. In cats, it was increased from 0.018 to 0.094 gamma per gram of fresh tissue. These values are lower than those observed in uteri of rabbits, in which the effects of estrogen are intense (Reynolds, 1939b; Reynolds and Foster, 1939). In view of the lability of acetylcholine in tissues, however, and in

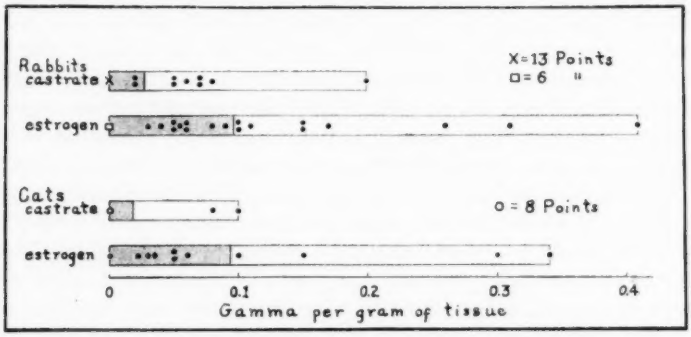

Fig. 1. Diagrams showing the mean concentrations of an acetylcholine-like substance (shaded bars) in extracts of nasal mucosa of ovariectomized rabbits and cats. Results from individual experiments are shown by dots. See text for discussion.

view of the small quantity and mixed character of the tissue used in the present work (mucosa, bone and cartilage), the results may properly be regarded as satisfactory proof of a cholinergic-like action of estrogen upon the nasal mucosa of both rabbits and cats.

A few experiments (five) were made with nasal mucosa from cats taken at the end of six hours after injection of estrogen. As has been found with the rabbit uterus, there is a diminution in the amount of an acetylcholine-like substance in the extracts. Two of the five were inactive, the remainder yielded concentrations of an acetylcholine-like substance of 0.04, 0.05 and 0.11 gamma per gram of fresh tissue, or an average for the group of 0.041 gamma per gram of fresh tissue.

SUMMARY

1. The effect of estrogen on the nasal mucosa is cited. It consists of moderate vasodilatation. Consideration of indirect evidence suggests

that it may be the result of a local increase in the quantity of free acetylcholine.

2. On this basis, comparison is made directly of the concentration of an acetylcholine-like substance in two groups of ovariectomized rabbits and cats, one of which is untreated, the other, after receiving a subcutaneous injection of estrogen.

3. It is found that both the number of extracts containing measurable amounts of an active substance, and the mean concentration of this material is increased one hour after injection of estrogen.

4. Attention is called to the fact that a similar cholinergic action of estrogen is demonstrable in the uterus of the rabbit, but not in the uterus of the cat. In the former, the uterine innervation is cholinergic; in the latter, it is adrenergic.

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EFFECT OF GELATINE FEEDING UPON THE STRENGTH AND FATIGABILITY OF RATS' SKELETAL MUSCLE

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Reports concerning the effect of gelatine upon fatigability, whether positive (Ray, Johnson and Taylor, 1939) or negative (Hellebrandt, Rork and Brogdon, 1940) have involved subjective criteria as to the point of fatigue. While such criteria may be valid for measurements of fatigue as a psychic reaction they do not necessarily reflect the functional capacity of the muscle. The object of the following experiments was to test, in an objective fashion, the effect of gelatine, fed as a supplement to an adequate diet, upon the functional ability of muscle.

TABLE 1

Effect of gelatine supplements upon skeletal muscle

SUPPLEMENT	NUMBER OF ANIMALS	TENSION-TIME	FATIGUE-TIME	MAXIMUM TENSION PER GRAM	CREATINE
		kgm. sec. per gram	*seconds*		*mgm. per 100 grams*
Gelatine	11	24.8	39	1,827	455
Glucose	9	24.2	39	1,761	450

Rats were used as the experimental animals. Adult males of four to six months of age from an inbred stock were matched as to size, and placed in two groups. Both were fed a stock diet of commercial dog biscuits. In addition, the individuals of one group were given ½ gram of gelatine in 3 cc. of water by stomach tube daily. The control group was similarly given ½ gram of glucose in 3 cc. of water. The supplements were continued for one week when the functional ability of one gastrocnemius of each of the rats was tested. For this, the animal was anesthetized with ether. The gastrocnemius was exposed and its tendon cut and attached to a torsion rod. The femur was rigidly fixed and the muscle stimulated directly with maximal induction shocks at the rate of 60 per second. The response was magnified with an optical lever and recorded on film. When the tension returned to the base line the stimulation was discontinued and the gastrocnemius removed from the animal,

weighed and analyzed for creatine. The tension curves were measured for 1, maximum strength; 2, the area of the tension curve to 10 per cent of maximum strength, and 3, the time to 10 per cent of the maximum strength.

The area of the tension curve, tension-time, has the same dimensions as work and is, therefore, proportional to the work capacity of the muscle. The data in the table show that there is no significant difference between the work capacities of the muscles of gelatine and glucose fed rats.

Fatigability is usually measured as the time from beginning of activity to the point of inability to respond. The time to complete lack of response is difficult to measure with accuracy. Therefore, the time required for the response to fall to 10 per cent of the maximum was measured. This fatigue time is identical for the two groups of rats.

The maximum strength of the two groups of muscles is not significantly different. This, taken with the similar areas for tension-time and identical fatigue times, indicates that the genesis of fatigue in the two groups is the same.

Analysis of the muscle showed, furthermore, that there were no changes in the average creatine concentration of the muscles of these two groups of animals.

The feeding time of one week was chosen as a period that should allow for such changes as reported by Ray, Johnson and Taylor, 1939. Because of their results the experiment was confined to male animals.

CONCLUSIONS

It may be concluded from these results that feeding of gelatine supplements to the normal adult male rat leads to no changes in the muscle itself which enhance functional performance.

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THE INFLUENCE OF MORPHINE ON TRANSPORTATION IN THE COLON OF THE DOG

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The correlation of normal activity and transportation in the colon of the unanesthetized, trained dog has already been studied (1). By letting the balloon serve as the bolus, it was possible to determine the relationship of transportation to the segments of the colon, periods of activity, sustained tonicity and even to the different phases of individual contractions. An efficiency gradient in which the greatest transporation occurs in the most proximal segments has been described as being dependent upon the character of contractions rather than upon the total quantity of activity. Transportation was found to be not only related to certain types of activity such as type II and III contractions but in the majority of instances to their systolic phases.

An extension of this work designed to study the force exerted by the colon in an attempt to move material to a lower level was measured by a technique as nearly isometric as possible (2). The pull pattern was found to be intimately related to the type II and III contractions and was only occasionally observed when the tone of the colon was extremely high and sustained for several minutes.

The effect of morphine on colon motility has been studied with the tandem balloon technique (3, 4). In these studies the augmenting effect of morphine upon the tone and activity of the colon was observed. The tremendous exaggeration of activity seen to follow the administration of moderate doses of morphine is not in accord with clinical observations that morphine is constipating, if the quantity of activity is to be considered the factor responsible for transportation in the colon. In other work (1, 2) it has been pointed out that transportation is more intimately associated with the character of activity rather than the quantity. Other observers have proposed that the constipating action is primarily related to an augmented sphincter activity which is far in excess of the augmentation occurring in other parts of the intestine. If this were the entire explanation one would expect a piling up of material in the best developed sphincter regions below the cardia, namely, at the pyloric, ileocecal and anal sphincters. However, while most observers are in accord in reporting

that this does occur at the pyloric sphincter no consistent findings have revealed an uneven distribution of material in other parts of the intestinal tract.

With the recording balloon used as a bolus free to move in the colon, the influence of morphine upon its movement was studied in a series of 15 experiments on 4 trained cecostomized dogs, following a series of 40 control experiments in which no morphine was used. The balloon bolus was inserted into the proximal colon by way of the cecostomy to a depth of 10 cm. beneath the skin and inflated according to a standard method (1). Before the injection of morphine 100 minutes of control motility and bolus transportation were recorded, thus making a total of 55 control periods of 100 minutes each (table 1). During the first and second 50 minutes of the 100 minute control period in 55 experiments the colon was active 48 to 56 per cent of the time and the balloon was observed to move approximately 4 cm. in each of the 50 minute divisions. During the first 50

TABLE 1

Effect of morphine on transportation

50 MINUTE PERIODS	1	2	3	4
*Minutes of control motility	25	28	27	27
Centimeters of control transportation	4.1	3.9	3.6	2.6
Minutes of motility after morphine			42	46
Centimeters of transportation after morphine			17	0.2

* **Fifty-five** experiments—40 = continued as controls; 15 = morphine injected at close of **2nd** 50-minute period.

minutes following the injection of morphine the activity of the colon segment occupied by the balloon was found to be increased to 84 per cent and transportation to 17 cm., while the control tracings for this same period in 40 experiments reveal an expectancy of activity to be 51 per cent and a transportation of only 3.6 cm. During the second 50 minute period which followed the injection of morphine the activity of the colon was higher (92 per cent) than that of the first 50 minutes, but the transportation was practically negligible, amounting only to 0.2 cm. during the entire period. These figures are in marked contrast to the transportation expectancy for this period suggested by the control experiments.

To study further the effect of morphine on the colon 15 experiments were conducted in which the force exerted by the colon to displace a bolus to a lower level was measured by an isometric apparatus previously described (2). From the data thus obtained it was evident that here too the character of activity is more important than quantity of activity. Simultaneous with the increase in activity elicited by morphine there was recorded an increase in transportation force. When the activity was sus-

tained either as high tone or exaggerated type I and type II contractions, as was the case in the majority of instances, the force tending to displace the balloon rapidly diminished. On the other hand, in those instances where high tone or type III contractions alternated with brief periods of relative quiet or exaggerated type II contractions the tendency to displace the balloon was more evenly distributed throughout the experiment and more conspicuously associated with rising tone or type III contractions.

These findings corroborate previous work (2) which has associated the pull pattern with types of activity and together with the data on transportation suggest that the tendency of the colon to transport material from one segment to another is dependent upon the type of activity in adjacent segments as well as the type of activity in the segment in which the bolus rests.

The tandem balloon technique has revealed certain periods after the injection of morphine in which the different segments of the colon are not in simultaneous activity. The character of activity at this time is very comparable to that of the activity recorded by the single balloon during the time when transportation and transportation force is being manifest. It is logical that constriction of any segment of the colon tends to prevent the passage of material through its confines from an adjacent segment even though it tends to empty itself of its own contents. It seems reasonable therefore that a segment of high pressure will be able to empty itself only if an adjacent segment is of lower pressure. The efficiency gradient (1) is therefore at least in part dependent upon the state of activity existing in adjacent segments. Normally this efficiency gradient tends to permit material to pass through the colon at a physiological rate. The injection of morphine augments the normal gradient immediately following its administration by exerting its first effects most profoundly upon the more proximal segments. Shortly after this immediate effect the extent of the morphine action is more evenly distributed to all parts of the colon, making each segment serve as a barrier to the passage of material through its limits. Thus all parts of the colon become physiological sphincters retarding the shifting of materials. This conception merely expands upon an older conception that augmented sphincter action at the anatomical sphincters accounts for the constipation following morphine administration.

It was found early in the work with the tandem balloon system that the colon of the dog may be divided into two physiological units not dependent upon anatomical divisions (5). These units, proximal and distal segments, are characterized by certain types of activity but the dividing line between them may shift from time to time. This division although usually in the region of the splenic flexure might shift to any part of the colon or might

disappear entirely, permitting the colon to act in its entirety as a proximal-like or distal-like unit. Since the proximal segment is characterized by pressure changes of the type II and type III variety directed in an aboral or oral manner and the distal segment is characterized mainly by type II simultaneous contractions, material is caused to shift more rapidly in the proximal than in the distal segments. This probably is a factor in explaining the well known clinical observations that the distal colon is more responsible for constipation than is the proximal. The character of activity observed in the second phase following morphine injection (retarded efficiency gradient) is more like that of distal colon activity in that pressure changes with directional character are largely replaced by strong type I and II activity of stationary character.

The authors are indebted to Dr. A. J. Carlson, who made this study possible.

SUMMARY

1. Following the administration of moderate doses of morphine to unanesthetized dogs, there was an immediate augmentation followed by a retardation of the rate of transportation of a bolus in the colon.

2. The force exerted by the colon to transport a bolus was measured by an isometric technique which revealed a close correlation between that force and the character of activity rather than the quantity of activity.

3. A dual effect of morphine is described as being first an augmentation and second a retardation of the normal "*efficiency gradient.*"

4. The phase of retardation in the normal "*efficiency gradient*" in which activity in the lower segments more nearly approaches that of the proximal segments in extent and character may account for constipation after morphine administration.

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THE DEVELOPMENT OF THE ANACROTIC AND TARDUS PULSE OF AORTIC STENSIS[1]

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Cardiac specialists have long possessed the empirical knowledge that marked stenosis of the aortic orifice is accompanied by characteristic changes in the radial pulse. There is a definite halt in the rise of pressure and the principal peak occurs perceptibly later in the cycle than it does in the normal pulse. These abnormalities can be shown in a radial sphygmogram, and in severe cases can be detected by expert palpation. Attempts to explain the hydrodynamic mechanisms underlying the association of these phenomena have stopped short of completeness. Katz, Ralli and Cheer (4) showed the changes in the central pulse produced in dogs by gradually tightening a ligature around the root of the aorta. Emphasis was laid upon a deep notch developing, with increasing stenosis, on the anacrotic limb as the probable source of the anacrotic halt in the radial pulse. Feil and Katz (1) presented paired simultaneous sphygmograms from subclavian and radial arteries of stenosis patients, showing associated abnormalities in the two pulses. No one has heretofore bound the two researches together with a study of the development of the peripheral from the central pulse during progressive stenosis of the aortic orifice. This report presents the results of experiments designed to fill that gap.

METHOD. Dogs were anesthetized with sodium barbital (250 mgm. per kgm. intravenously) after a small dose of morphine. Under artificial respiration the chest was opened wide in the mid-line and the heart was suspended in a cradle made of the opened pericardium. A stout loop (a round shoe lace) was passed around the root of the aorta and fastened to a device with a calibrated screw, with which any desired degree of stenosis could be obtained and reproduced (used by Katz, Ralli and Cheer). The central pressure pulse was obtained by the use of a six-inch cannula inserted into the left common carotid artery and pushed down until its tip was in the ascending aorta just distal to the ligature. This cannula, like all the rest, was connected by lead tubing to a high-frequency ma-

[1] Preliminary report, This Journal **129**: P 347, 1940.

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nometer of the Gregg type, the rubber-membrane modification of the Hamilton manometer, recording photographically at a distance of about two meters.

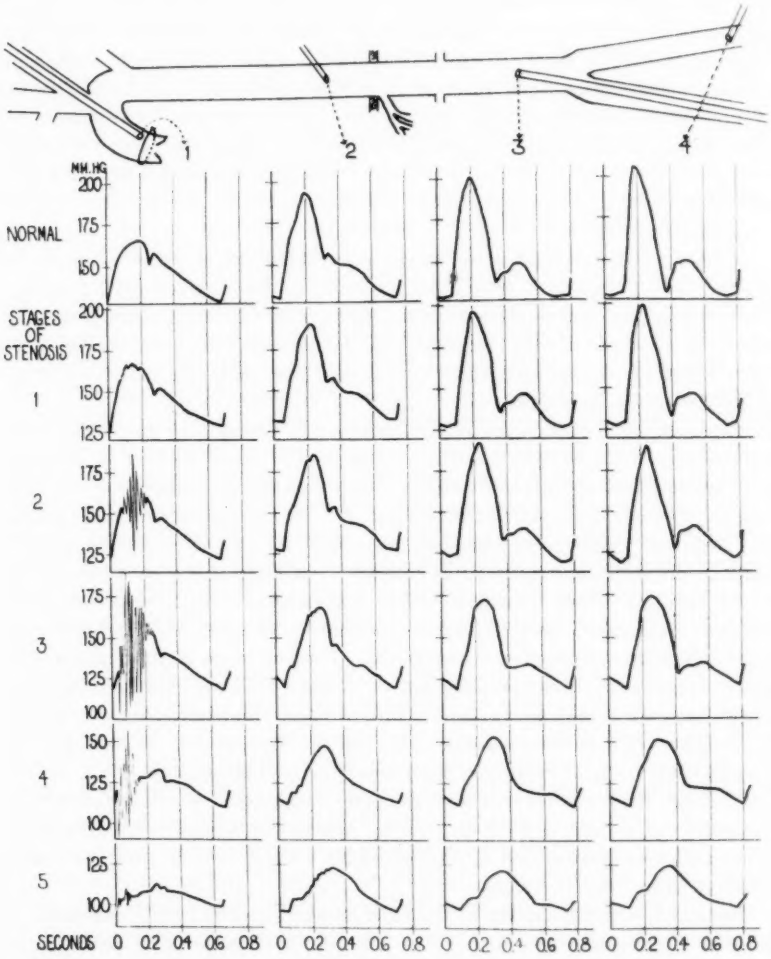

Fig. 1. Pressure pulses recorded simultaneously (each horizontal row) from four different parts of the arterial system with gradually increasing aortic stenosis (reading downward). Note that in each vertical column zero time indicates the start of the pulse in the ascending aorta.

In several experiments, various peripheral pressure pulses were obtained by the method of Hamilton and Dow (3), using a long cannula run up from

the right femoral with its tip movable to any desired point in the descending aorta-iliac-femoral system. At each of several positions of this cannula, a series of paired simultaneous pressure pulses was obtained for each of ten or more degrees of stenosis. While much valuable information was obtained by this method, it was sometimes difficult to compare the results quantitatively because of small changes in the central pulse from one series to the next. Therefore, in the experiment reported here, the tip of the long cannula was left just above the bifurcation, the aorta was punctured with a needle cannula just above the diaphragm, and a needle was inserted in the left femoral artery. This arrangement furnished four simultaneous pressure pulses from different parts of the system at each stage of the stenosis.

Results. Reconstructions, to the same time and pressure scales, of a representative series of records are shown in the accompanying figure. Each horizontal row depicts the pressure pulses obtained simultaneously at the four designated arterial points at the indicated stage of stenosis. The following features of these pressure curves may be pointed out as pertinent to the problem.

The findings of Katz, Ralli, and Cheer on the changes in the pulse at the root of the aorta with stenosis are confirmed. Most obvious is the production of a mid-systolic turbulence when the orifice is narrowed partially but not enough to diminish the discharge. The breakdown of the smooth ejection into turbulence is signaled, as in their records, by a sharp anacrotic notch. As stenosis becomes more nearly complete, the systolic discharge is severely cut down, with the result that after the third stage the turbulence diminishes and finally disappears, and systolic, diastolic, and pulse pressures all decrease markedly. Less obvious at first glance but easily measurable is a prolongation of systole with no change or even a slight increase in the heart rate. The peak of this aortic pulse occurs a little later than in the control, but only in proportion to the systolic lengthening. The incisura is flattened almost to extinction.

It should be observed that two marked changes take place in the fundamental form of the central pulse: the initial rise becomes progressively lower and less violent, and it is followed by a short plateau merging into a gradual ascent to the peak.

The most striking change in the form of the other pulses (columns 2, 3, 4) is a progressive decrease in the amount of modification with transmission. The four pulses are quite different when there is no stenosis, but they become more like each other and like the central pulse through a gradual collapse of a high peak early in the cycle. It should be noted that except for a few cases in the thoracic aorta they are all smooth and show no transmission of the vibrations of the central turbulence.

Discussion. The only concept, essential for the conversion of the

foregoing observations into an explanation of the radial pulse of aortic stenosis, is Otto Frank's (2) likening of the peripheral pulse to a slow manometer's recording of the central pulse. Such a manometer distorts both form and size of rapid pressure fluctuations because of the inertia of the fluid and its momentum when set into periodic oscillations. The degree of distortion depends upon the magnitude of pressure changes which are too rapid to be followed faithfully.

In the above experiments it can be seen that the stenosis restrains the impact of the usual sudden ejection into the aorta to such an extent that the peripheral pulse is permitted to reproduce quite faithfully the fundamental form of the central pulse. The resistance to flow at the stenosis has meanwhile molded the central pulse into just the form actually found at the radial in clinical cases.

The tardus quality of the stenotic pulse can be seen in the figures to be due to the disappearance of an early peak rather than to the introduction of a late one. Hamilton and Dow's (3) identification of the principal peak of the femoral pulse as a standing wave rested upon the determination that it occurred simultaneously everywhere beyond a point of phase reversal near the aortic arch. The peripheral pulse, therefore, starting later, shows its principal peak sooner after its start than does a central pulse. When the ejection is not sufficiently violent to set up this standing wave, the remaining later peak is merely the representation of that in the central pulse, formerly obscured by the superimposed wave. This disappearance can be followed, reading downward in each column of the figure, until only the fundamental central pulse form is left, modified on the anacrotic limb by the restraint which the stenosis imposes on the ejection.

In an unpublished experiment (shown before the American Physiological Society at Washington, 1936) Dow and Hamilton demonstrated the importance of the factors mentioned above. In a dog's aorta, in situ but completely tied off, relatively slow pressure changes, even though large, impressed at the cardiac end, were reproduced faithfully at the bifurcation. Even small sharp impacts, on the contrary, were distorted in form and magnitude by the periodic oscillations or standing waves set up.

This analysis agrees in many ways with that of Feil and Katz. It departs from that of Katz, Ralli and Cheer in minimizing the importance of the sharp downward break in the central pulse at the start of the turbulence as the source of the anacrotic halt in the radial pulse. In these experiments the two phenomena seem quite independent. The "anacrotic incisura," caused by the sudden breakdown of smooth flow into turbulence, reaches a maximum with moderate stenosis, then decreases in size; while the anacrotic plateau, due to interference with ejection, continues to grow in prominence.

It is true that the present experiments do not correspond in detail to the clinical picture explained by them. The development of the femoral rather than the radial pulse has been chosen for its accessibility. However, the same dynamic factors are operative in both cases and any difference can be only a quantitative and not an essential one. As was pointed out by Katz, Ralli, and Cheer, the artificial stenosis beyond the valves rather than at them permits better maintenance of the coronary flow than is possible in the clinical case. Perhaps this feature compensates for the rapid induction of the stenosis with no opportunity for other mechanisms to become useful.

Consideration of the simultaneous pulse in the thoracic aorta suggests that the diminution of pulse pressure in the ascending aorta may be somewhat exaggerated by recording the central pulse in the turbulent stream right at the orifice. Although the cannula pointing toward the heart should pick up the total pressure (lateral plus velocity), it is possible that the tip may have been pushed to one side, out of the direct stream and into an eddy pocket.

SUMMARY

The anacrotic halt and tardus characteristics of the radial pulse in cases of aortic stenosis are shown to be due to the following factors:

1. Stenosis so reduces the violence of the systolic discharge that standing waves are not set up and the peripheral pulse reproduces the central pulse form with almost complete faithfulness.

2. The stenosis offers so much resistance to flow during mid-systole that the central pulse itself assumes the anacrotic and tardus characteristics.

Acknowledgment is gratefully given to Dr. Carl J. Wiggers for the suggestion of this problem and for kindly advice and assistance. Thanks are also due to Messrs. J. H. Geyer and B. S. Brown for technical aid in several experiments.

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GRADED PARTIAL PANCREATECTOMY

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Ceccherelli (1) cited the early work of Brunner in 1682 as first demonstrating that partial pancreatectomy did not impair the health or digestion of the experimental animal. Total pancreatectomy, however, is incompatible with life unless some form of substitution therapy is employed.

In 1939 we (2) reported from this laboratory a series of experiments demonstrating that in dogs in which a large portion of pancreas had been removed, degenerative changes in the liver could be prevented if the transected end of the remaining pancreas was transplanted into the stomach, thus preserving its external secretion. It has been observed repeatedly by numerous investigators that partial pancreatectomy does not disturb, to any degree, the sugar metabolism. We have interested ourselves in attempting to determine how much of the pancreas could be removed before the health of the animal was impaired as determined by blood sugar, blood amylase, plasma prothrombin determinations and microscopical studies of liver biopsies.

METHOD. Healthy mongrel dogs of both sexes, weighing between 6 and 15 kgm., were used in this experiment. They were kept on a diet of milk and bread, supplemented with Karo, yeast and cod liver oil.[1] After varying control periods, during which determinations of the blood sugar, blood amylase and plasma prothrombin were made, partial pancreatectomy was performed. Varying amounts of the gland were removed. At the time of operation, a liver biopsy was taken, and this repeated at weekly intervals for the first postoperative month. At approximately 60 and 90 days postoperative, other liver biopsies were taken, and again at postmortem examination. The values of the blood sugar, blood amylase and plasma prothrombin were determined at the time the liver biopsies were taken, or in some animals, more frequently during the first week after operation.

The blood sugar was determined by Benedict's (3) method. The blood

[1] Milk and bread in the ratio of 4 quarts to 3 loaves. Karo—3 tablespoons to each quart of the above mixture. Yeast—3 teaspoons to each quart of the above mixture. Cod liver oil—1 tablespoon to each quart of the above mixture.

amylase was done by a slight modification of the procedure described by Elman (4). To 5 cc. of a colloidal suspension of 2 per cent soluble starch, 1 cc. of plasma and 2 cc. of 0.9 per cent sodium chloride were added. This was done in duplicate for each blood specimen. On one of the resulting mixtures a sugar determination was run *immediately* (without filtration). The other was incubated for 30 minutes at 37.5°C. before the sugar content was determined. The difference between the non-incubated and incubated specimens represents the amylase value in terms of the milligram per cent sugar produced by the action of the serum amylase on the starch solution. The sugar in the non-incubated specimen was found to be slightly higher than the combined values of the blood sugar and starch blank.

The plasma prothrombin was done by the method described by Warner, Brinkhous and Smith (5).

At the time of death, the remnant of pancreas was weighed, and from this figure was calculated the per cent of the gland which had been removed.

Results. The gland remnants were weighed at the time of postmortem examination. It was found that a rather regularly graduated series had been obtained in which the amount of the pancreas removed varied from 17 to 84 per cent. The individual proportions of removed tissue were 17, 19, 24, 48, 53, 63, 66, 71, 73 and 84 per cent.

Sugar. In none of the dogs was diabetes produced, except in the animal from whom 84 per cent of the pancreas was removed. In all the others, the blood sugar values did not vary beyond the normal range. The animal which developed diabetes died two weeks after operation. The blood sugar rose to 273 mgm. per cent one week after operation and reached 426 mgm. per cent three days before death. The blood sugar taken on the ninth postoperative day showing 102 mgm. per cent came after a three day fast.

Amylase. The blood amylase values varied over a wide range both during the control and postoperative periods. In each animal the fluctuation was marked. For convenience of statistical analysis, three groups were formed. In the first group were the animals from whom 17, 19 and 24 per cent of the pancreas had been removed. In the second group were those animals who lost 48, 55, 63 and 66 per cent of the pancreas at operation; and in the third group those from whom 71, 73 and 84 per cent of the gland had been removed. The amylase values were analyzed preoperatively and postoperatively within each of these groups.

It is evident that the difference in pre- and postoperative means (table 1) is well below the minimum difference necessary for significance. The same is true when the postoperative means of the various groups are compared. These figures seem to indicate that the removal of varying pro-

portions of the pancreas, up to 84 per cent, had no significant effect on the blood amylase level.

Prothrombin. The plasma prothrombin was not significantly altered by the removal of varying portions of the pancreas. The greatest variation was from 80 to 100 per cent, but this occurred in only one dog (no. 8). In four others, the variation was from 86 to 100 per cent, while in the remainder it was between 90 and 100 per cent.

Liver biopsy. The liver biopsies removed at weekly and later monthly intervals after operation showed no conspicuous change from the normal. In the animal with 66 per cent of the pancreas removed (no. 7), the sections of liver removed on the twenty-first and twenty-eighth postoperative

TABLE 1

Statistical data for amylase values in different groups

	MEAN	S.D.	P.E.	MINIMUM DIFFERENCE FOR SIGNIFICANCE	ACTUAL DIFFERENCE
Group I					
Preoperative	529	172	41	147	31
Postoperative	560	152.5	21		
Group II					
Preoperative	567	159	27.6	108	74
Postoperative	493	159	19.6		
Group III					
Preoperative	570	171	34.6	138	75
Postoperative	495	164	25.5		

S.D., standard deviation; P.E., probable error.

days showed slight fatty degeneration, which, however, was not evident in the sections removed 59 and 118 days postoperative. The only other evidence of fatty change in the liver was found in the tissue removed from dog 8 (71 per cent of pancreas removed) on the thirteenth postoperative day. None of the sections of liver from the animal with 84 per cent of the pancreas removed showed any fatty degeneration.

SUMMARY

1. Portions of the pancreas, varying from 17 to 84 per cent, were removed from ten dogs, and their courses followed with blood sugar, blood amylase and plasma prothrombin determinations. Liver biopsies were examined at definite intervals postoperatively.

2. Only in the animal from whom 84 per cent of the pancreas had been removed did diabetes develop. All other blood sugar values were within normal limits.

3. Blood amylase values varied over a wide range both pre- and post-operatively. Statistical analysis of the data reveals no significant change in blood amylase values caused by removal of from 17 to 84 per cent of the pancreas.

4. Plasma prothrombin values were not affected by partial pancreatectomy.

5. The liver biopsies showed, in general, no conspicuous change from the normal. Fatty degeneration was present transiently in the two animals from whom 66 and 71 per cent of the pancreas had been removed. In none of the other biopsies was fatty degeneration seen.

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HORMONAL FACTORS AFFECTING THE SURVIVAL OF ADRENALECTOMIZED MICE[1]

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Synthetic progesterone and the hormones from ovaries heavily luteinized by pretreatment with gonadotropins have been shown to ameliorate the symptoms of adrenal insufficiency in rats (1–7), cats (8, 9), and ferrets (10), whereas male hormone is ineffective in this respect (4) or even harmful (11). If a similar differential effect of these substances obtains in mice, adrenalectomy should constitute a technique for distinguishing between large amounts of endogenously produced progesterone and androgen as, for example, following prolonged treatment of female mice with large doses of pregnant mare serum (12). This procedure cannot at present be utilized because of the paucity of information available concerning the effects of the different sex hormones upon the adrenalectomized mouse. With the object both of acquiring this needed information and of determining the effects of other sex hormones as well as of an adrenal cortical hormone in a species thus far largely neglected in this respect, the present study was undertaken.

MATERIAL AND METHODS. A total of 195 adrenalectomized mice of the A (Strong) strain were used in these experiments. Adrenalectomy was performed as a single stage operation, using the bilateral lumbar approach with a single dorsal skin incision. The adrenal vessels were not ligated, but instead were pinched off well behind the adrenal with a small curved forceps in such a manner that the adrenal was removed with the surrounding fat. To rule out the factor of personal differences in operative technique, all the adrenalectomies were performed by the same operator. Castration was performed by the usual techniques. In all the operations the animals were anesthetized with ether. When both castration and adrenalectomy were performed, a period of at least 10 days intervened between the two operations.

[1] Supported in part by the Rockefeller Foundation and the Fluid Research Fund of Yale University School of Medicine.

The synthetic hormones[2] used in this investigation were estradiol benzoate, testosterone propionate, progesterone and desoxycorticosterone acetate. These hormones were dissolved in sesame oil in such concentration that the daily dose was contained in 0.05 cc. of oil. Excessive luteinization of the ovary was obtained by treatment with pregnant mare serum.

TABLE 1

Survival time of controls, sesame oil treated, and desoxycorticosterone acetate treated adrenalectomized "A" mice

TREATMENT	NUMBER AND SEX OF ANIMALS	AGE	DAILY DOSE	DRINKING WATER	NUMBER OF ANIMALS DYING IN LESS THAN		
					10 days	15 days	15 days
					From beginning of treatment		After cessation of treatment
		days					
Controls	10 ♀	120		2% saline	10		
	5 ♂‡	40		2% saline	3	2	
	5 ♀‡	40		2% saline	4†		
Sesame oil	5 ♂	45	0.5 cc.	2% saline	4	1	
	5 ♀	45	0.5 cc.	2% saline	3	1†	
	5 ♂‡	40	0.5 cc.	2% saline	5		
	5 ♀‡	40	0.5 cc.	2% saline	5		
Desoxycorticosterone acetate	8 ♂‡	52	0.25 mgm.	2% saline	7		1
	10 ♀	180	0.25 mgm.	Tap water			10
	4 C_3H* ♂	90	0.25 mgm.	2% saline	2		2
	5 NH* ♀	150	0.25 mgm.	2% saline	5		
	3 C_3H* ♂	90	0.25 mgm.	Tap water			3
	6 NH* ♀	150	0.25 mgm.	Tap water	1		5

* These two strains of mice were included only to eliminate the possibility that the toxicity of desoxycorticosterone acetate and saline might be strain limited and are, therefore, not listed in "Material and Methods."

† The other animal of this group lived indefinitely due to the presence of accessory adrenal tissue.

‡ Castrated.

The P.M.S.[3] was a water solution and as much as 0.1 cc. was injected in some cases. All hormone injections were started two days before ad-

[2] The estradiol benzoate, testosterone propionate and progesterone were generously supplied by the Schering Corporation through the courtesy of Dr. E. Schwenk. The Ciba Pharmaceutical Products Company, through the courtesy of Dr. E. Oppenheimer, kindly furnished the desoxycorticosterone acetate.

[3] Purified pregnant mare serum "Gonadin" was generously supplied by Cutter Laboratories through the courtesy of Drs. Donald Wonder and C. Parham.

renalectomy and administered daily unless otherwise noted. The diet in all cases consisted of Purina Fox Chow. The drinking water of the adrenalectomized animals contained about 2 per cent sodium chloride except in the few cases listed in table 1.

RESULTS. At the beginning of these experiments untreated adrenalectomized animals survived from 2 to 6 days, but later the minimal survival time increased appreciably. This increase in survival time is ascribed to an improvement in operative technique with consequent diminution of trauma and shock. Throughout the experiment the majority of the animals died before the seventh day. Animals which survived longer than 10 days often lived indefinitely, possibly as a result of the hypertrophy of accessory adrenal tissue. For these animals to survive, it was necessary to continue the saline for 2 or 3 weeks. Desoxycorticosterone acetate was used as a known means of maintaining life after adrenalectomy (table 1). This substance in a daily dose of 0.25 mgm. protected all the adrenalectomized mice which did not receive NaCl in the drinking water. When given saline, however, 7 of 8 animals receiving desoxycorticosterone acetate survived less than 10 days. The saline group chewed at their wounds, often removing a considerable area of skin around the incision. They also showed an increased tendency to eat any animal which died, usually devouring everything but the bones and skin. Edema was observed in those few animals available for autopsy. The effects of desoxycorticosterone acetate and saline were not peculiar to animals of the A strain since similar behavior was noted in mice of the NH and C_3H strains. The fact that some of the C_3H mice survived may have been due to their greater body size since they weighed 28 grams as compared with 20 and 18 grams body weight in the A and NH strains, respectively.

As shown in table 2, progesterone was effective in maintaining life in adrenalectomized mice. Daily injection of 0.5 mgm. maintained life in about half of the animals while 1 mgm. protected completely against the effects of adrenalectomy throughout the period of injection. Upon termination of progesterone injections, all animals died of adrenal insufficiency within the expected time. Saline was given throughout these experiments.

Notwithstanding the protective action of injected progesterone, corpora lutea of pseudopregnancy and of pregnancy were of doubtful effectiveness in this respect. Of 10 mice adrenalectomized 3 to 5 days after copulation with a vasectomized male, only one lived more than 10 days, and this one lived indefinitely, possibly due to hypertrophy of accessory tissue. The corpora lutea of the operated animals showed signs of atrophy, indicating that adrenalectomy affected their normal function. Other female mice adrenalectomized on the twelfth day of pregnancy, or when the placental

sign was first observed, usually died within the range of survival of the untreated, adrenalectomized controls, although 3 animals which either resorbed their fetuses or aborted survived slightly longer.

Female mice which received 20 r.u. of pregnant mare serum daily for 35 days preceding adrenalectomy survived somewhat longer than those re-

TABLE 2

The effectiveness of progesterone and of functional corpora lutea in ameliorating the symptoms of adrenal insufficiency in adrenalectomized "A" mice

TREATMENT	NUMBER AND SEX OF ANIMALS	AGE	DAYS OF PRE-TREATMENT*	TOTAL DAYS TREATED	DAILY DOSE	NUMBER OF ANIMALS DYING IN LESS THAN		
						10 days	15 days	10 days
						After adrenalectomy		After cessation of treatment
		days						
Progesterone	5♂**	65	2	12	1.0 mgm.			5
	5♀**	65	2	12	1.0 mgm.			5
	5♂**	60	2	12	0.5 mgm.	3		2
	5♀**	60	2	12	0.5 mgm.	2		3
Pseudopregnancy	10♀	120				9†		
Pregnancy	10♀	120				7	3	
P.M.S.¶	3♂**	100	65	‡	5 r.u.	3		
	5♂**	100	65	‡	10 r.u.	5		
	10♀	70	35	‡	20 r.u.	4§		
	8♀	70	35	‡	20 r.u.	4§		
	8♀	105	75	‡	20 r.u.	4	4	
	10♂	95	65	‡	20 r.u.	10		

* Number of days before adrenalectomy that hormone treatment was begun.

† The other animal of this group lived indefinitely due to the presence of accessory adrenal tissue.

‡ Treatment was continued until the animals died or it became clear that they would survive indefinitely.

§ The remaining animals in this group survived indefinitely but no evidence of accessory adrenal tissue was visible macroscopically.

¶ The dosage of P.M.S. (pregnant mare serum) is expressed in terms of the Cole-Saunders rat unit which is biologically equivalent to two of the international units recently established.

** Castrated.

ceiving 0.5 mgm. progesterone. The ovaries of these animals showed excessive luteinization and were probably secreting large amounts of progesterone. However, when the P.M.S. was continued for 75 days, the protective action of the ovary became very slight. By this time much of the luteal tissue of the ovary had been replaced by luteal-like cells and apparently progesterone production had fallen below the level needed to protect against adrenalectomy. However, when adrenalectomized animals

were protected by the heavily luteinized ovaries for 10 to 25 days, removal of the ovaries did not prevent their subsequent survival. That this survival was not due to a direct action of the pregnant mare serum is shown by its failure to protect intact males and castrates which had received the same P.M.S. treatment.

The action of the other sex hormones known to be secreted by the stimulated ovary were also tested (table 3). A single dose of 1 γ of estradiol benzoate did not affect the survival time. In daily doses of 1 γ or higher, however, this substance was toxic and caused the death of all the adre-

TABLE 3

Effects of various doses of estradiol benzoate and of testosterone propionate upon the survival time of adrenalectomized "A" mice

TREATMENT	NUMBER AND SEX OF ANIMALS	AGE	DAYS OF PRE-TREATMENT*	TOTAL DAYS TREATED	DAILY DOSE	WEEKLY DOSE	NUMBER OF ANIMALS DYING IN LESS THAN		
							5 days	10 days	20 days
							After adrenalectomy		
		days							
Estradiol benzoate	5♀	50	0	1	1.0 γ		2	3	
	5♂	35	2	†	1.0 γ		4	1	
	5♀	35	2	†	1.0 γ		5		
	10♀	180	10	1	16.6 γ		3	7	
	10♂	85	35	28		50.0 γ	8	2	
	10♀	85	35	28		50.0 γ	10		
Testosterone propionate	4♂	75	2	†	2.5 mgm.‡			3	1
	4♀	75	2	†	2.5 mgm.‡			2	2

* Number of days before adrenalectomy that hormone injections were begun.

† Injections were continued until the animals died.

‡ A 14 mgm. pellet of testosterone propionate was implanted subcutaneously at the time injections were begun.

nalectomized mice within 5 days. Pretreatment with 16.6 γ estradiol benzoate weekly until the week before adrenalectomy also shortened the survival time. This effect was evidently due to a residue of the injected estrogen in the body at the time of adrenalectomy as shown by the non-effect of a single injection of estrogen given 10 to 30 days before adrenalectomy. Both immature and mature females were used in the latter experiment to control any possible effect of the corpora lutea which form in estrus, but not in immature, female mice following a single injection of estrogen. These corpora lutea, like those of pseudopregnancy, were ineffective. Testosterone propionate did not shorten the survival time

even when injected in daily doses of 2.5 mgm. in addition to a 14 mgm. pellet implanted subsequent to the adrenalectomy. All the pellets became infected in the adrenalectomized mice, although such an occurrence was not observed in intact animals. The survival time of the mice treated with testosterone propionate was possibly slightly increased but probably not sufficiently to be significant. There was no evidence of any sex difference in response to any of the hormones tested.

Discussion. It is evident from the above results that the production of progesterone and male hormone by the mouse ovary can be distinguished by adrenalectomy. The observation that progesterone in sufficient quantity will protect against the fatality of adrenalectomy in mice is in agreement with the results which have been obtained with other species studied (4, 5, 6, 7, 9, 10). However, the fact that between 0.5 mgm. and 1.0 mgm. of progesterone is required for this protection makes the mouse agree more nearly with the rat (4, 5, 6, 7) than with the ferret (10) or cat (9) if the differences in body weights are taken into account. The protection appears to be of a different character from that provided by desoxycorticosterone acetate in that the progesterone does not markedly affect the salt and water balance (4), while desoxycorticosterone acetate, if administered in appreciable quantities (0.25 mgm. daily) in conjunction with increased salt intake, causes sufficient salt and water retention to be fatal. However, mice treated with desoxycorticosterone acetate survive readily on a normal diet and salt intake (13). Similar observations have been recorded for man (14).

The fact that 35 days' pretreatment with P.M.S. protects against adrenalectomy, while pretreatment for 75 days gives only a slight increase in survival time over that of the controls, indicates that up to 35 days appreciable quantities of progesterone are being produced by the heavily luteinized ovaries, whereas by 75 days progesterone production has diminished below the level required for maintenance of life in the adrenalectomized mouse. This is apparently associated with the replacement of the true luteal tissue by luteal-like cells and the production of appreciable quantities of male hormone (12). It is of interest, however, that adrenalectomized animals which have been maintained for some time by the luteinized ovaries may be castrated and still survive. Since no accessory tissue was discovered, although all possibilities of its presence have not been exhausted, it may be that the survival is due to an adjustment of the organism to the lack of adrenal cortical hormone (15, 16) which takes place during the period that the animal is maintained by the luteinized ovary.

The mouse seems to differ from some of the other species (8, 10, 17, 18) in that pseudopregnancy and pregnancy do not protect against the consequences of adrenalectomy. This absence of protection indicates that

even though the ovary can be stimulated to produce adequate amounts of progesterone to protect against adrenal insufficiency, it either does not do so under normal conditions or the period of great activity of the corpora lutea is too short to be effective.

In agreement with the findings on other animals thus far tested (2, 19, 20, 21), estrogen in appreciable amounts is very toxic in adrenalectomized mice. In a minimal estrus-producing dose, however, estrogen has neither harmful nor beneficial effects. This observation is in agreement with certain results which have been obtained with rats (22). Male hormone has been reported to be both toxic (4) and noneffective in adrenalectomized rats (11). However, in the present experiment with mice, testosterone propionate, even in the large doses employed (2.5 mgm. daily plus a 14 mgm. pellet), was probably not beneficial, but was clearly not harmful.

No significant difference was observed between oil-treated and uninjected adrenalectomized mice even though sesame oil has been reported to contain a principle which prolongs life in adrenalectomized pregnant rats (23).

SUMMARY AND CONCLUSIONS

Adrenalectomized normal and castrated mice of the A strain survive 3 to 7 days. Administration of testosterone propionate is not harmful to adrenalectomized mice and is probably not helpful, while estradiol benzoate in any but the most minimal dose is toxic. Progesterone in 1 mgm. daily doses maintained life in all of 10 adrenalectomized mice treated; 5 of 10 animals survived on 0.5 mgm. daily. Desoxycorticosterone acetate was effective in ameliorating the symptoms of adrenal insufficiency, but was very toxic even in doses of 0.25 mgm. if given in conjunction with 2 per cent salt in the drinking water. Pregnant mare serum was ineffective in males and castrates, but pretreatment of females for 35 days before adrenalectomy luteinized the ovaries to such an extent that the survival period fell between that following 0.5 mgm. and 1.0 mgm. doses of progesterone. Longer periods of pretreatment (75 days) were less effective. Pregnancy and pseudopregnancy had little if any protective action. It can be concluded, therefore, that progesterone in sufficient amounts, whether produced by the ovary or injected, protects against the fatality of adrenalectomy in mice and that adrenalectomy may be used to distinguish between the endogenous production of large amounts of progesterone and male hormone.

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THE EFFECT OF ETHER ANESTHESIA UPON CERTAIN BLOOD ELECTROLYTES[1]

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The well-known decrease in blood CO_2 capacity produced by ether anesthesia has been studied by many investigators. Henderson and his co-workers (1, 2) have supposed that the respiratory excitement induced by ether caused an alkalosis by the blowing off of CO_2. It is suggested that under these conditions alkali passes into the tissues. Other investigators (3, 4, 5, 6) have reached a different conclusion. They find that the decrease in CO_2 capacity is accompanied by an equally rapid fall in plasma pH and by increase in CO_2 tension. Coincident with these events lactic acid appears in the blood in amounts sufficient to account in large part for the acidosis (6). More recently, Henderson (7) has restated his original contention.

In view of these facts it has seemed desirable to investigate the major electrolyte changes which occur during ether anesthesia.

METHODS. Dogs which had received water but no food since the previous day were placed on the animal board for two hours before the experiments were started. Ether was administered by the drop method and anesthesia was maintained for approximately one hour at such a depth that the corneal reflex was just absent. Complete narcosis with a minimum of struggling was attained within two minutes. Blood samples were drawn without stasis from the jugular veins.

The CO_2 capacity. The CO_2 content of oxalate-fluoride treated whole blood equilibrated at 38°C and 40 mm. Hg pressure CO_2 was determined by the method of Van Slyke and Neill (8).

Lactate. Whole blood was treated with oxalate and fluoride in the usual manner. Lactic acid was measured by Friedemann, Cotonio and Shaffer's (9) method.

Sodium and chloride. Plasma sodium was analyzed according to Butler and Tuthill (10) and plasma chloride was determined by Eisenman's technic (11).

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RESULTS. In seven experiments on five dogs, ether anesthesia for one hour caused an average decrease of 5.4 mM. in the CO_2 capacity. Table 1 shows that there was considerable variation in the bicarbonate decrease

TABLE 1

Effect of ether anesthesia on the CO_2 capacity and the lactic acid content of whole blood

(CO_2 capacity of dog's whole blood measured at 38.1° ± 0.1°C., pCO_2 = 40 mm. Hg; H_2CO_3 = 1.1 mM/L.)

All figures in mM/liter

DOG NUMBER	PERIOD OF ANESTHESIA			RECOVERY PERIOD		
	Duration of anesthesia	$BHCO_3$	Lactic acid	Duration of recovery	$BHCO_3$	Lactic acid
	min.			*min.*		
1	0	19.7				
	7	15.2				
	23	14.9				
	43	13.6				
2	0	19.0				
	15	14.7				
	32	15.6				
	62	13.5				
2	0	17.4	1.9	199	16.9	2.0
	11	14.4	3.6			
	59	10.8	6.4			
3	0	17.4				
	22	13.7				
	45	13.0				
	59	14.0				
3	0	15.9	0.6	60	13.4	5.2
	10	15.5	1.9			
	60	11.1	6.8			
4	0	14.9	1.8	118	14.0	2.1
	10	7.9	6.9			
	57	8.8	6.9			
5	0	12.5	1.3	36	12.5	3.8
	10	10.0	7.4			
	60	7.1	7.1			

which ranged between 3.4 and 6.6 mM. Even in experiments carried out upon the same animal (2 and 3) at different times the agreement was only qualitative. It is of particular interest to note that in general the CO_2

capacity decreased progressively with the duration of anesthesia. In those experiments in which this was not the case the aberrant points may be ascribed to a decrease in the depth of narcosis. Approximately two-thirds of the fall in the CO_2 capacity which was present at the end of one hour of anesthesia occurred during the first ten minutes.

The effect of ether anesthesia upon the blood lactic acid concentration was determined in four experiments (table 1). At the end of one hour the average increase in lactic acid was 5.4 mM. This value ranged in different experiments between 4.5 and 6.2 mM.

While the increase in blood lactate concentration may be larger or smaller than the decrease in CO_2 capacity in any given blood sample, the average increase in blood lactate after 10 minutes' etherization was 3.5 mM. in four experiments, which agrees well with an average decrease in bicarbonate of 3.2 mM. At the end of one hour these values were 5.4

TABLE 2

The relation between the diminished alkaline reserve and increased concentration of lactic acid during ether anesthesia

DOG NUMBER	AT 10 MINUTES		AT 1 HOUR	
	$BHCO_3$ decrease	Lactic acid increase	$BHCO_3$ decrease	Lactic acid increase
	mM/liter	*mM/liter*	*mM/liter*	*mM/liter*
3	0.4	1.3	4.8	6.2
2	3.0	1.7	6.6	4.5
4	7.0	5.1	6.1	5.1
5	2.5	6.1	5.4	5.8
Average.....	3.2	3.5	5.7	5.4

mM. for lactic acid and 5.7 mM. for bicarbonate (table 2). Although it is recognized that other changes in the acid-base balance have occurred, these results indicate that the increase in lactate is of the proper order of magnitude to account for the observed fall in CO_2 capacity.

Analyses made at varying periods after the anesthesia (table 1) showed that the excess lactate may be removed within one and a half hours. There was a simultaneous increase in the bicarbonate concentration.

In eight experiments plasma sodium and chloride analyses were made before, at the end of one hour of anesthesia, and approximately one hour after the withdrawal of ether (table 3). There was no significant change in the concentration of either electrolyte.

Since the withdrawal of appreciable amounts of blood was necessary for the various analytical procedures employed, hemorrhage might be assumed to be a factor in the results obtained. This point is particularly pertinent for it is known that the excessive loss of blood decreases the

alkali reserve and increases the blood lactate (12, 13 and others). Two control experiments in which every step except etherization was repeated are shown in table 4. Since the concentrations of bicarbonate and lactic

TABLE 3

Electrolyte analyses before, during and after one hour of ether anesthesia

DOG NO.	Na^+ IN MEQ. PER LITER OF SERUM					Cl^- IN MEQ. PER LITER OF SERUM				
	Control	Ether	Difference	Recovery	Difference	Control	Ether	Difference	Recovery	Difference
6	153.2	157.0	+3.8	152.4	−0.8	110.2	109.0	−1.2	108.4	−1.8
7	148.5	149.6	+1.1	146.7	−1.8	111.0	110.0	−1.0	113.2	+2.2
8	150.4	148.6	−1.8	149.0	−1.4	108.6	106.8	−1.8	106.9	−1.7
3	143.9	142.0	−1.9	141.0	−2.9	117.8	119.4	+1.6	118.1	+0.3
2	146.1	149.5	+3.5	145.3	+0.8	118.1	120.3	+2.2	120.2	+2.1
9	144.1	145.6	+1.5	146.3	+2.2	117.8	118.4	+0.6	118.9	+1.1
5	150.3	150.8	+0.5	149.8	−0.5	120.0	121.0	+1.0	119.0	−1.0
4	149.0	149.5	+0.5	149.5	+0.5	119.5	120.0	+0.5	121.0	+1.5
Average..			+0.9		−0.5			+0.2		+0.3

TABLE 4

Control experiments—no anesthesia

(CO_2 capacity of dog's whole blood measured at 38.1 ± 0.1°C., pCO_2 = 40 mm. Hg; H_2CO_3 = 1.1 mM/L.)

DOG	WEIGHT	TIME	$BHCO_3$	LACTIC ACID	BLOOD DRAWN
	kgm.	*min.*	*mM/L.*	*mM/L.*	*cc.*
10	20.0	0	17.2	2.2	20
		29	18.0	2.8	16
		94	18.2	3.0	20
		192	17.7	3.0	16
					72
11	13.1	0	14.8	1.6	25
		29	15.6	2.3	15
		98	15.0	2.8	17
		197	16.0	2.2	20
					77

acid are relatively unchanged, it can be assumed that the above data are not complicated by the effects of hemorrhage.

Discussion. According to Lipow, Weaver and Reed (14) the administration of ether to dogs generally decreased the blood sodium concentration during the first hour. Following this there was a pronounced increase. Fay, Andersch and Kenyon (15) found that one hour of ether anesthesia

increased the serum sodium slightly (2 to 3 m.-eq.). Similar values were reported by Marenzi and Gerschman (16) who concluded that these were of no signficance. Our determinations indicate that any changes in serum sodium during one hour of ether anesthesia are within the error of the method.

In three experiments in which dogs were etherized for twenty minutes Austin et al. (5) reported increases of 3.4, 2.5 and 5.9 mM. of chloride per liter of serum. Fay and her co-workers (15) state that one hour of ether anesthesia in dogs reduces the serum chlorides from 0 to 7 m.-eq. Like Reiman and Sauter (17) and Marenzi and Gerschman (16), we have concluded that ether anesthesia produces no significant change in the serum chloride concentration.

Assuming that sodium and chloride do not readily enter the cells (18), the failure to detect any significant change in the concentration of these ions lends support to the contention of one of us (19) that ether does not appreciably alter the volume of extracellular fluid. If there had been any movement of fluid in or out of the cells one might have expected to find a reciprocal change in serum sodium and chloride concentration.

Our experiments confirm the work of previous investigators (1, 2, 3, 4, 5, 6, and others) who have reported that ether anesthesia decreases the blood CO_2 capacity. Ronzoni et al. (6) showed that the fall in CO_2 capacity could be accounted for largely by the increased blood lactate. That the blood lactate increases in dogs during ether anesthesia has been confirmed by Collazo and Morelli (20), Fuss (21), and Fay, Andersch and Kenyon (15). Whereas the above experiments show blood lactate concentrations which are greater than those reported by Fuss (21) and Fay et al. (15), they are in excellent agreement with Ronzoni's (6) results.

The rate of disappearance of the blood lactate and the restoration of the CO_2 capacity following ether administration was more rapid in our experiments than in those reported by Ronzoni et al. (6) and by Cullen et al. (4). Since our results depend upon only a few experiments we do not attach much importance to this difference.

We are indebted to Dr. G. W. Thorn of the Johns Hopkins Hospital for help with the sodium and chloride analyses.

CONCLUSIONS

1. The effect of one hour of ether anesthesia upon the serum sodium, the serum chloride, the blood lactate and the blood CO_2 capacity of dogs has been studied.

2. One hour of etherization produces no significant change in the serum sodium and the serum chloride concentrations.

3. The decrease in CO_2 capacity (4.8 to 6.6 mM.) present at the end of

one hour of ether administration can be accounted for largely by the increase in blood lactate concentration (4.5 to 6.2 mM.).

4. After ether anesthesia the return of the lactate and the CO_2 capacity to the control values occurs in about one and one-half hours.

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CALCIUM IN THE COAGULATION OF THE BLOOD[1]

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It is generally accepted that thrombin is formed from the interaction of prothrombin, thromboplastin and calcium, but much uncertainty remains concerning the manner in which calcium functions in this reaction. In the present paper the rôle of this element is studied under conditions in which the other two components of the reaction are made constant. It has been shown by the author (1) that the prothrombin is relatively constant for any one species. Thromboplastin can easily be made a constant by adding an excess of the reagent. For this study chicken blood is well adapted since it can be maintained fluid without the addition of any anticoagulant, thereby avoiding the introduction of outside factors other than thromboplastin itself.

Blood was drawn from the wing vein into a syringe which had been chilled with ice, and transferred into collodion-coated test tubes which were immersed in an ice bath. Doctor Hirschboeck (2) has recently discovered that collodion is more efficient in retarding coagulation than paraffin and this observation has been repeatedly verified in the writer's laboratory. The plasma was obtained by centrifuging the blood in an angle centrifuge at 3,000 r.p.m. This plasma at the temperature of the refrigerator remained fluid for several weeks. For the preparation of thromboplastin 0.1 gram of chicken brain (dehydrated with acetone according to the author's method for rabbit brain (3)) was mixed with 5 cc. of 0.85 per cent sodium chloride solution and incubated for 20 minutes at 50°C.

To determine quantitatively the effect of thromboplastin on the clotting time, 0.1 cc. of plasma was mixed in a small test tube with 0.2 cc. of saline solution containing varying concentrations of the thromboplastin emulsion. The tube was put into a water bath kept at 37½°C., and the clotting time recorded with a stop watch. The results are presented in figure 1. It will be noted that as the amount of thromboplastin was progressively increased, the clotting time rapidly decreased until a certain concentration was reached, after which the rate remained constant irrespective of the excess

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of thromboplastin. This finding is exceedingly important since it enables one to make the thromboplastin factor a constant merely by adding enough of the agent to obtain the minimal optimum concentration. The experiment presents strong evidence that the absence or delay in the coagulation of chicken blood is probably due solely to a lack of thromboplastin, and it furthermore demonstrates that an exceedingly small amount of this agent is sufficient to bring about coagulation in the time which is considered within the normal range.

It is fairly certain that circulating blood does not contain free or available thromboplastin. This agent is apparently liberated only after the blood is shed; and the rate at which it becomes available determines the

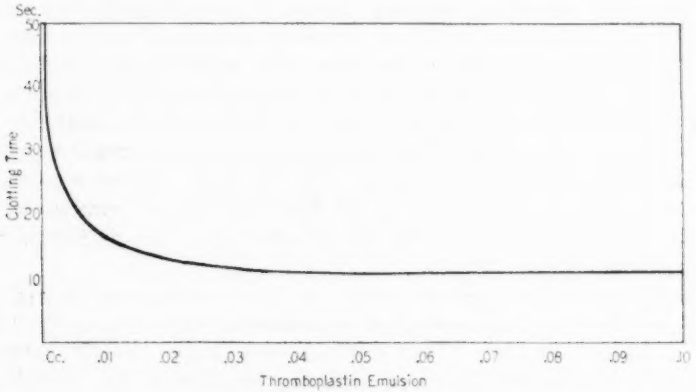

Fig. 1. The relationship of the clotting time to the concentration of thromboplastin. For the determination, 0.1 cc. of chicken plasma (containing no decalcifying agent) was mixed with 0.2 cc. of physiological saline solution containing varying amounts of thromboplastin emulsion.

coagulation time. Three important factors must be considered in the liberation of thromboplastin: 1, species; 2, temperature; and 3, surface of container. In regard to species it is well known that goose blood, if not contaminated with thromboplastin from tissue juices, will remain fluid in an ordinary glass container. Likewise horse blood will not clot in a glass receptacle provided it is kept in an ice bath. Human and rabbit bloods on the contrary coagulate rapidly even when cooled in ice, but the low temperature nevertheless has a demonstrable retarding effect on the clotting time of these bloods. The third factor, the surface of the container, is of utmost importance. Freund (4) as early as 1886 recorded that a coating of vaseline retarded coagulation, and later Bordet and Gengou (5) demonstrated that paraffin was better and more convenient. It has now been found that collodion is even superior to paraffin (2). The comparison

of the effect of glass and collodion on coagulation is shown in table 1. From these results it can be seen that coagulation is greatly retarded in a collodion-coated tube and that less oxalate is required to maintain the fluidity of human and rabbit blood. These differences in clotting are due without doubt to variations in the liberation of thromboplastin. Ferguson (6) as well as earlier investigators have shown that calcium is an important factor in the lysis of platelets, and thus it seems logical to assume that even partial removal of the calcium of the blood will decrease the rate of platelet destruction with the result that less thromboplastin is liberated. Excess

TABLE 1

The influence of the surface of the container on the amount of sodium oxalate required to inhibit coagulation

SPECIES	CONTAINER	SODIUM OXALATE 0.1 M ADDED TO 5 CC. OF BLOOD						
		0	0.05 cc. (0.001 M)*	0.075 cc. (0.0015 M)	0.10 cc. (0.004 M)	0.15 cc. (0.003 M)	0.20 cc. (0.004 M)	0.25 cc. (0.005 M)
Goose	Glass	No clot						
	Collodion	No clot						
Chicken	Glass	Clot	Clot	Clot	Clot	No clot		
	Collodion	No clot						
Man	Glass	Clot	Clot	Clot	Clot	Clot	No clot	
	Collodion	Clot	Clot	No clot				
Rabbit	Glass	Clot	Clot	Clot	Clot	Clot	Clot	No clot
	Collodion	Clot	Clot	Clot	Clot	Clot†	No clot	

The blood was drawn into a cold syringe, mixed immediately with sodium oxalate, centrifuged, and then placed in a refrigerator.

* The figures in parentheses indicate the calculated molar concentration obtained by diluting the sodium oxalate to 5 cc.

† A few threads of fibrin appeared after several hours.

oxalate will in addition remove the calcium needed for converting prothrombin to thrombin, but it is obvious that the clotting time of blood furnishes no significant information concerning the fraction of calcium which actually takes part in the coagulation reaction.

The calcium required for converting prothrombin to thrombin. In order to study successfully the calcium factor in coagulation, it is necessary to make the other constituents of the reaction, namely, prothrombin and thromboplastin, constants. Since the concentration of prothrombin for any one species is fixed (1), only thromboplastin needs to be considered. It has already been shown, in figure 1, that this can easily be done by adding an excess of this reagent. Therefore the problem actually resolves

itself into determining how much calcium must be removed before coagulation is inhibited in the presence of an optimal concentration of thromboplastin. This is readily accomplished by adding varying amounts of sodium oxalate to a fixed amount of blood, and then determining the coagulation time of the plasma after an excess of thromboplastin has been added. The results of this experiment on chicken and human bloods are presented in table 2.

Several conclusions can be drawn from these results. It is clear that if just sufficient sodium oxalate is added to precipitate the total calcium of the blood, coagulation is not inhibited. To prevent clotting 0.2 cc. of

TABLE 2

The quantity of sodium oxalate required to inhibit coagulation in the presence of excess thromboplastin

	SODIUM OXALATE 0.1 M ADDED TO 5 CC. OF BLOOD						
	0	0.05 cc. (0.001 M)*	0.075 cc. (0.0015 M)	0.10 cc. (0.002 M)	0.20 cc. (0.004 M)	0.30 cc. (0.006 M)	SPECIES
	Clotting time in seconds						
2 hours after adding sodium oxalate	11	11	13	22	180	No clot	Chicken
10 min. after adding sodium oxalate	11	11	16	45	No clot	No clot	Man
1 hour after adding sodium oxalate		11½	45	†	No clot	No clot	Man

The clotting time was determined by mixing 0.1 cc. of the oxalated plasma with 0.1 cc. of physiological saline solution, and 0.1 cc. of thromboplastin emulsion.

* The figures in parenthesis indicate the calculated molar concentration resulting from diluting the sodium oxalate to 5 cc. Blood containing 6 mgm. of calcium per 100 cc. of whole blood has a 0.0015 M concentration of calcium. Therefore the addition of 0.075 cc. of 0.1 M sodium oxalate to 5 cc. of blood supplies the molar equivalency of the calcium content.

† A few shreds of fibrin formed after one hour.

0.1 M sodium oxalate must be added to 5 cc. of blood. At this dilution the sodium oxalate is 0.004 M. Since the concentration of calcium is approximately 0.0015 M (6 mgm. per 100 cc. of blood), it can be concluded that somewhat less than 3 times the amount of sodium oxalate required to precipitate the total calcium is necessary to prevent coagulation in the presence of an optimal concentration of thromboplastin.

It is to be further noted that the action of sodium oxalate is not instantaneous. To study this time factor more accurately, whole blood is not particularly suitable. Better adapted for this study is plasma containing no anticoagulant agent, but maintained fluid by keeping it in a collodion-coated test tube cooled by means of ice. The experiment was

performed by mixing 0.5 cc. of plasma with varying amounts of sodium oxalate, and after definite time intervals, taking 0.1 cc. of the oxalated plasma, mixing it with 0.1 cc. of saline and 0.1 cc. of thromboplastin emulsion and then determining the clotting time. The results which were obtained are summarized in table 3. The important fact brought out by this study is that the anticlotting action of sodium oxalate is not immediate, but requires a measurable period of time. The higher its concentration, the shorter the period required to bring about complete inhibition of coagulation. Again it is found that an amount of oxalate equivalent to approximately 3 times the total calcium of the plasma (10 mgm. per 100 cc. of plasma) will, if allowed to react for several minutes before

TABLE 3

The time factor in the inhibition of coagulation by sodium oxalate

TIME OF INCUBATION	SODIUM OXALATE 0.1 M ADDED TO 0.5 CC. OF PLASMA (CONTAINING NO DECALCIFYING AGENT)						
	0	0.01 cc. (0.002 M)	0.02 cc. (0.004 M)	0.03 cc. (0.006 M)	0.04 cc. (0.008 M)	0.05 cc. (0.010 M)	
	Clotting time in seconds						
10 sec.	11	11½	12	16	17	20	Chicken
1 min.		12	14	75	300	No clot	plasma
5 min.		12½	35	360	No clot	No clot	
30 min.		12½	140	660	No clot	No clot	
10 sec.	11	11	12	12	14	20	Human
1 min.		10½	17	165	300	No clot	plasma
5 min.		*	*	No clot	No clot	No clot	

The clotting time was determined by mixing 0.1 cc. of the oxalated plasma with 0.1 cc. of saline solution and 0.1 cc. of thromboplastin emulsion.

* Plasma clotted spontaneously.

thromboplastin is added, prevent completely coagulation though an excess of the latter factor is present. Interestingly, when 0.02 cc. of 0.1 M sodium oxalate is added to 0.5 cc. of chicken plasma, the retarding action on coagulation is slow but definitely progressive, and at the end of 30 minutes the clotting time is increased from 11 to 140 seconds. The same amount of oxalate added to human blood likewise begins to inhibit coagulation, but before 5 minutes elapse, enough thromboplastin is liberated, presumably from the platelets, to cause spontaneous coagulation. It is easy to see that an amount of sodium oxalate sufficient to inhibit ultimately coagulation, is inadequate if during this period thromboplastin is liberated. Obviously consistent results cannot be obtained unless the thromboplastin factor is controlled, and in the light of this it is easy to understand why conflicting findings were frequently obtained in the past.

Certain practical considerations arise from the present work. It is often observed that blood containing an adequate amount of oxalate or citrate will, nevertheless, on standing contain a film or veil of fibrin. If such blood be used for transfusions, the danger of emboli is encountered. No satisfactory explanation for this slight coagulation has been given, but on the basis of the present findings it seems reasonable to suppose that the fundamental cause is the slow decalcifying action of oxalates and citrates. During the time the active calcium is removed, sufficient thromboplastin may become liberated to convert a small amount of prothrombin to thrombin and although only a minute quantity of the latter may be formed, it is sufficient to clot enough fibrinogen to form a visible film of fibrin. To prevent this incipient coagulation, it is not only necessary to employ a definite excess of sodium citrate or oxalate, but also to prevent the liberation of thromboplastin. Among the means to accomplish the latter are: chilling the blood; using chemically clean containers (perhaps substituting a plastic such as Lampert's athrombit (7) for glassware); and avoiding air bubbles and foaming. It appears certain that the interface, air-blood, is an underestimated factor in liberating thromboplastin.

Certain conclusions concerning the action of calcium can be drawn from the present results. It appears certain that ionized or free calcium does not take part in the conversion of prothrombin to thrombin. If it were the unbound calcium, the anticlotting action of sodium oxalate would be immediate since the removal of the ionized calcium would not require the time interval observed in the experiments of table 3. The most logical interpretation of the findings recorded in this study is that prothrombin itself contains calcium, and that like proteins is weakly dissociated or ionized. In blood one finds the equilibrium:

$$\text{Prothrombin Ca} \rightleftharpoons \text{Prothrombin}^{=} + \text{Ca}^{++}$$

It is easy to understand why an equivalent amount of sodium oxalate does not inhibit coagulation for the insoluble calcium oxalate likewise dissociates:

$$\text{Ca oxalate} \rightleftharpoons \text{Ca}^{++} + \text{oxalate}^{=}$$

Consequently as long as there is a sufficient concentration of calcium ions, the prothrombin complex remains intact. From the law of mass action one knows that:

$$\frac{\text{Ca}^{++} \times \text{oxalate}^{=}}{\text{Ca oxalate}} = K_1$$

and

$$\frac{\text{Ca}^{++} \times \text{prothrombin}^{=}}{\text{Prothrombin Ca}} = K_2$$

An equilibrium is thus established between the two systems. From table 3 one can deduce that if slightly less than 0.02 cc. of 0.1 M sodium oxalate is added to 0.5 cc. of chicken plasma enough calcium ions remain in solution to preserve the prothrombin Ca complex. If, however, more oxalate is added to plasma, the calcium ions are depressed and at a certain concentration the calcium is completely torn from the prothrombin molecule. At this point, the prothrombin is no longer able to react with thromboplastin and therefore coagulation is completely inhibited. According to this concept prothrombin in its normal state as it occurs in the blood is a calcium compound which is converted to thrombin as soon as it reacts with thromboplastin.

In oxalated plasma, no true or normal prothrombin occurs but only an inactive decalcified derivative. On recalcifying oxalated plasma, prothrombin is quickly regenerated according to the following equation:

$$\underset{\text{(Inactive form)}}{\text{Prothrombin}} + \text{Ca} \rightarrow \underset{\text{(True active form)}}{\text{Prothrombin Ca}}$$

In contrast to decalcification which is a relatively slow process, recalcification is practically instantaneous indicating that prothrombin has a great avidity for calcium. This is strikingly illustrated by a rather simple series of experiments. By mixing plasma containing a fixed excess of oxalate with thromboplastin and then adding calcium chloride solution, optimal coagulation was obtained for a wide range of calcium concentrations as shown in table 4. Theoretically, a calcium chloride concentration of 0.0075 M will precipitate all of the oxalate contained in the plasma, but curiously a concentration as low as 0.00065 M is still able to cause clotting. From 0.0025 to 0.025 M the clotting time is fairly constant and corresponds rather closely to the rate observed after adding thromboplastin to plasma containing no anticoagulants. This suggests that for these concentrations of calcium, the prothrombin is quickly and completely regenerated and will cause clotting in the period normally observed for a fixed amount of prothrombin. Naturally, for low or inadequate amounts of calcium not all of the prothrombin can be reformed. For higher levels of calcium the depressing action of this ion (8) begins to manifest itself.

If calcium chloride is added first to the oxalated plasma, followed by thromboplastin one minute later, strikingly different results are obtained. Weak solutions of calcium, i.e., below 0.0025 M, no longer are sufficient to cause clotting, and even higher concentrations are inadequate to bring about clotting within the normal time of 11 seconds. But a definite excess of calcium such as is obtained by adding a 0.02 M solution will cause clotting in about 7 seconds. This can be easily explained. By incubating oxalated plasma with excess calcium chloride, the thromboplastin of the plasma has a chance to react with the prothrombin before the

thromboplastin emulsion is added, and therefore some thrombin is already present before the main reaction occurs with the result that the clotting time is definitely shortened. By mixing oxalated plasma with an inadequate amount of calcium chloride before adding thromboplastin, a distribution of the calcium can take place with the establishment of an equilibrium of the calcium held by the oxalate, the proteins and the prothrombin. Thus the amount of prothrombin Ca which exists for any particular equilibrium determines the coagulation time.

One important practical deduction to be made from this study is that the method of determining quantitatively prothrombin by the clotting time

TABLE 4

Effect of the concentration of calcium chloride on the clotting time when added to oxalated plasma containing an excess of thromboplastin and when allowed to react with oxalated plasma before thromboplastin is added

MOLAR CONCENTRATION OF CALCIUM CHLORIDE	CLOTTING TIME IN SECONDS	
	Plasma mixed with thromboplastin before calcium chloride was added	Thromboplastin added 1 minute after plasma was mixed with calcium chloride
0.250	11½ to 12	7 to 8
0.200	11 to 12	7 to 8
0.015	11 to 12	11 to 12
0.010	11 to 11½	13 to 23
0.005	10 to 11	60 to 200
0.0025	10 to 11	300 to 500
0.00125	13 to 17	No clot
0.00062	60 to 90	No clot

For the determination 0.1 cc. of plasma, 0.1 cc. of thromboplastin, and 0.1 cc. of calcium chloride were employed.

One cubic centimeter of 0.1 M sodium oxalate was added to 9 cc. of blood. On the basis that whole blood contains 6 mgm. per 100 cc., the sodium oxalate concentration of the plasma in excess of the calcium is approximately 0.0075 M.

of recalcified plasma containing an excess of thromboplastin has for its support the significant observation that the clotting time of normal plasma to which an excess of thromboplastin is added is essentially the same as that of oxalated plasma which is mixed with thromboplastin and a fixed quantity of calcium chloride. This observation has not only been made on chicken and human bloods, but also on the bloods of the horse, rabbit, dog, and other animals.

The conclusion that it is the combined calcium which is responsible for the conversion of prothrombin to thrombin is not new. The fact that to prevent clotting 3 times more oxalate must be added than is required to precipitate the total calcium as Vines, (9) Scott and Chamberlain (10) have found has led Vines and Collingwood (11) to conclude that the free or

ionized calcium does not enter into the coagulation mechanism. Recently Ferguson (12) obtained evidence that a calcium containing intermediary complex is formed during the conversion of prothrombin to thrombin. It is difficult to see from his data how one could differentiate between normal prothrombin which according to these present studies is a calcium compound, and this intermediary complex. From the data present, one must conclude that the calcium is already bound to the prothrombin before it reacts with thromboplastin. After the completion of this paper, Martin (13) published the results of a study on the action of various decarboxylic acids on the clotting time. He likewise reached the conclusion that prothrombin is a calcium compound and he postulates that prothrombin in its conversion to thrombin is first decalcified and then acted upon by ionized calcium. This differs from the writer's conclusion that prothrombin is directly converted to thrombin by means of thromboplastin without ionized calcium.

SUMMARY

1. Addition of increasing quantities of thromboplastin to native chicken plasma (not decalcified) causes a progressive increase in the speed of coagulation until a fixed minimal time is reached after which the rate remains constant irrespective of the excess of thromboplastin.

2. The speed with which blood (uncontaminated with tissue juice) coagulates is determined by the rate with which thromboplastin is liberated. Among the important factors influencing the latter are: 1, species; 2, temperature; and 3, surface of container. The more the liberation of thromboplastin is inhibited, the less the amounts of decalcifying agents such as oxalates and citrates needed to prevent coagulation.

3. To determine the absolute amount of sodium oxalate required to inhibit coagulation, specimens of blood containing increasing known amounts of oxalate are tested by mixing a definite volume of the plasma with a fixed excess of thromboplastin. Approximately 3 times the amount of oxalate calculated to precipitate the total calcium is required to prevent the clotting of chicken and human bloods.

4. The anticoagulating action of sodium oxalate is not immediate. The greater the excess of oxalate the faster the coagulation is inhibited. By testing plasma with excess thromboplastin after definite time intervals following the addition of oxalate, no inhibition is found if slightly less oxalate is added than the amount calculated to precipitate all the calcium. In the case of human blood an amount of oxalate approximately 2½ times the calculated calcium equivalent of the plasma will inhibit clotting completely within 5 minutes whereas a 4-fold excess stops clotting in less than 1 minute.

5. Oxalated plasma containing excess thromboplastin will on recal-

cification clot promptly, and for a wide range of calcium chloride concentrations coagulation will occur in approximately the same time as is observed for unoxalated plasma mixed with excess thromboplastin.

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THE EFFECT OF ADRENALECTOMY AND OF FASTING ON THE FUNCTIONAL CAPACITY OF THE RAT'S GASTROCNEMIUS

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Although muscular weakness has long been observed to accompany corticoadrenal insufficiency, it is not clear to what extent such incapacity of the muscle may be due to a deficiency within the muscle itself, and to what extent nervous and circulatory factors may be responsible for the apparent muscular inadequacies. The observations of Hartman and Lockwood (1) on the fatigability of the synapses and myoneural junction in adrenalectomized rats seem to indicate that the nervous system is affected to a sufficiently profound degree to account for much if not all of the observed asthenia of adrenal insufficiency. Ingle (2–5) has demonstrated that muscles of adrenalectomized animals, when directly stimulated three times a second, cease to respond sooner than do those of normal animals, and do less work during the period that they do respond. Under the conditions of his experiments, however, a normal muscle continues to contract for many days, and in the case of an adrenalectomized animal the muscle stops contracting only when the whole animal collapses. The length of time during which the muscle continues to contract under these conditions, as well as the amount of work which it does, does not necessarily bear a relationship to the condition of the muscle at the beginning of the experiment, but may be merely a reflection of the ability of the circulatory system to maintain adequate conditions for the recovery of the muscle between contractions. Circulatory disturbances, possibly profound enough to account for his results, are a well known feature of adrenal insufficiency. We have therefore reinvestigated the effect of adrenal insufficiency on muscle function by causing the muscle to contract maximally and continuing the stimulation at such a rate that the muscle would remain in complete tetanus until fatigued. Since under these conditions the muscle has little time for recovery, it is believed that the total performance will more nearly reflect the condition of the muscle itself.

METHOD. Female rats of seven to eight weeks of age were used. Adrenalectomy was performed by the usual dorso-lateral approach at one stage. Muscle tension experiments were performed when the adrenal-

ectomized animals showed signs of adrenal insufficiency as judged by weight loss. The controls were matched for age and weight with the experimental animals, and were from the same litters whenever practicable. They were normal in every particular except that a sham adrenalectomy was performed; to control the factor of inanition which accompanies adrenal insufficiency, another group had no operation, but were fasted to the same weight loss as occurred in the adrenalectomized rats.

In a tension experiment, the animal was anesthetized with ether, the femur fixed vertically in a clamp, and the tendon of the left gastrocnemius attached to a Blix type of torsion rod of medium resistance, with optical recording which gave a magnification of 322 to 1. Maximal stimuli were delivered from the secondary of an inductorium at the rate of 60 a second through electrodes in contact with the muscle. Stimuli were continued and the isometric response continuously recorded until it was judged that the muscle was no longer exerting tension. The gastrocnemius was then dissected out and weighed.

OBSERVATIONS. Forty-eight animals were used in these experiments, 16 in each group. The maximum tension was developed early within the first second of stimulation, and usually the tension began to decline almost immediately, rather rapidly during the first twenty-five seconds, and then more and more slowly, so that near the end of the record it was very difficult to judge with certainty exactly when the base line was reached. For this reason we have arbitrarily considered the muscle "fatigued" when the tension declined to 10 per cent of the maximum. The "fatigue time," then, is the time from the beginning of stimulation to the point where the muscle is exerting only 10 per cent of its original maximum tension, keeping the rate and intensity of the stimulation constant throughout.

The maximum tension exerted by the sham-operated rats averaged 1977 grams per gram of muscle; the average for the fasted rats was 2084 grams per gram, and for the adrenalectomized rats, 1862 grams per gram. Since the standard errors $\left(\frac{\sigma}{\sqrt{n}}\right)$ for these three averages were 66, 84 and 72, respectively, it is evident that there was no significant difference in the absolute strengths of the muscles of the various groups of animals. The only other measurement of the absolute strength of muscles from adrenalectomized animals of which we are aware was made by Gans and Miley (6), by a method of trial and error, hanging weights on a Harvard type muscle lever, and determining how great a weight the muscle would lift when stimulated by single shocks. They also concluded that adrenalectomy did not reduce the absolute strength of the rat's gastrocnemius.

The average fatigue time for each group of muscles was as follows: sham-operated rats, 46.2 seconds; fasted, 36.1 seconds; adrenalectomized, 32.5 seconds. The differences between these means seem to be of a

considerable order of magnitude, but they are of doubtful significance, since the standard errors of the means are 4.1, 2.1 and 3.0, respectively.

Although the maximum strength of the muscles from adrenalectomized rats was as great as that of the muscles from the other groups, and the fatigue time was only slightly shorter, nevertheless their performance was distinctly inferior. This fact is evident from an inspection of table 1, which shows that during the early course of the stimulation the tension declines more rapidly in the adrenalectomized group than in the others, so that by the end of the fifth second it is significantly lower, and becomes increasingly so until about the fifteenth second. From that point on, the three curves run approximately parallel, although the tension exerted by the adrenalectomized group remains significantly lower than the others. The decline in tension in the fasted group roughly parallels that of the sham-operated group throughout, so that at no time during the stimulation is there any significant difference in the tension exerted by the muscles of these two sorts of animals.

The inferior performance of the muscles of adrenalectomized animals is clearly brought out when one calculates the average "tension-time" for each group. The unit of tension-time may be taken as the kilogram-second, and is defined as the maintenance of a tension of one kilogram for a period of one second. It is calculated as follows:

$$\text{tension-time in kilogram-seconds per gram} = \frac{T_1 + T_2 + T_3 + \cdots T_n}{w}$$

T_1, T_2, etc., represent the average tension in kilograms developed by the muscle during the first second, second second, etc., of stimulation; T_n is the time when the tension is 10 per cent of the original maximum; w is the weight of the muscle in grams. The tension-time is an expression of the area under the tension curve as recorded by the optical kymograph. The values obtained for the three groups of muscles, together with their standard errors, are as follows: sham-operated, 33.8 ± 2.1; fasted, 31.2 ± 1.6; adrenalectomized, 22.3 ± 1.6.

Discussion. From these results it would seem that adrenal insufficiency does not reduce the functional ability of rat's skeletal muscle for very brief bouts of exercise, but does adversely affect its capacity if the muscle is called upon for a sustained maximal effort. This deficiency is a functional one, for adrenalectomy does not result in a specific atrophy of the muscle; in fact, in the present series of animals, the ratio of gastrocnemius weight to total body weight was exactly the same in both the normals and the adrenalectomized animals; namely, 0.53 gram per 100 grams. The physical or chemical changes in the muscle which might account for the observed functional loss are unknown. Deficiency in carbohydrate stores in the muscle can not account for the reduced capacity, for Britton and Silvette (7) and Silvette (8) have shown that in rat muscle

adrenalectomy does not result in greater reduction of muscle glycogen than does simple fasting. Furthermore, if glycogen storage greatly affected the functional capacity of the muscle, one would expect the muscular performance of fasted rats to be inferior to that of normals, but this is not the case (table 1); it has been established (9) that fasting results in some degree of glycogen depletion in rat's gastrocnemius.

TABLE 1

Effect of adrenalectomy and of fasting on the response of rat's gastrocnemius muscle to tetanizing stimuli

TIME AFTER BEGINNING OF STIMULATION	CONTROLS TENSION	FASTED		ADRENALECTOMIZED	
		Tension	Difference from controls	Tension	Difference from controls
seconds	*grams per gram*	*grams per gram*		*grams per gram*	
1	1944	2075	131 ±97*	1837	107 ±95*
2	1881	2025	144 ±82	1763	118 ±85
3	1819	1969	150 ±79	1663	156 ±77
4	1675	1863	188 ±71	1481	194 ±72
5	1556	1725	169 ±64	1331	225 ±69
10	1144	1163	19 ±55	825	319 ±69
15	863	800	63 ±55	537	326 ±57
20	625	612	13 ±59	369	256 ±59
25	494	450	44 ±65	294	200 ±63

* Standard error.

Whatever the explanation for the reduced performance of the muscle of adrenalectomized animals, it would appear to be a defect present within the muscle before the beginning of stimulation.

SUMMARY

Optical records were obtained of isometric contractions of the gastrocnemius muscles of normal, adrenalectomized and fasting rats. Induction shocks at the rate of 60 a second were used as stimuli, and the response was continuously recorded from the beginning of stimulation until fatigue. The maximum tension exerted at the beginning of the response was the same for all the groups of animals, and the muscles from the adrenalectomized rats fatigued only a little more quickly than the others. The total performance of the adrenalectomized animals, however, was distinctly inferior to the others, because the tension declines more rapidly during the early part of the period of stimulation. It is believed that the functional inferiority of the muscles from adrenalectomized rats reflects a deficiency which existed within the muscle itself prior to the beginning of the stimulation, because under these conditions the muscle is called upon to perform maximally during the entire period of response, and the

level of performance is therefore not dependent upon the efficiency of the circulatory supply during the period of stimulation. The performance of muscles from fasting animals is not inferior to that of the controls; therefore, the functional deficiency observed after adrenalectomy is not due to the general inanition of the organism which accompanies adrenal insufficiency.

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THE EFFECT OF FLUID ABSORPTION ON THE DILUTION INDICATOR TECHNIQUE OF GASTRIC ANALYSIS[1, 2]

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The dilution indicator technique of gastric analysis was recently described in detail and a study was made of the reliability of the various concentrations estimated by this method (Hollander and Glickstein, 1940) with phenol red as the dilution indicator. In another article we reported the results of an application of this method to several human subjects with no obvious gastric pathology, wherein the phenol red test-meal contained alcohol (3.5 per cent or 7 per cent), caffeine solution (6.7 mgm. per liter), or distilled water (Penner, Hollander and Post, 1940). In these studies with humans it was found that the observed concentrations of acid and chloride did not differ significantly from those usually reported in ordinary fractional analyses, but the corrected concentrations were frequently above 165 mN (which corresponds approximately to the tissue fluids in tonicity) and sometimes were even several times this value. Similar hypertonic values have been reported by Wilhelmj, O'Brien and Hill (1936) who ascribed their occurrence to water absorption by the stomach but pursued the matter no further. Since the cause of these hypertonic values may prove to be a major complicating factor in future applications of the dilution indicator technique, we decided to investigate them further and to consider what procedure may be devised to eliminate their occurrence — if this be at all possible.

METHOD. All experiments were performed on dogs in essentially the same way, except for the type of test-meal. The fasting animal was supported in a specially constructed box, a Rehfuss tube was passed into the stomach, fasting contents were removed, the stomach was washed with 50 cc. of the test-meal, and 200 cc. of fresh test-meal were introduced through the stomach tube. Every 15 minutes thereafter the stomach was evacuated completely, the contents mixed, the volume recorded, and all but 15 cc. returned through the tube. This procedure was continued until

[1] This investigation was supported in part by a grant from the Friedsam Foundation.

[2] A preliminary report of this work was presented in the Proc. Am. Soc. Biol. Chem., J. Biol. Chem. **128:** xlvii, 1939.

no more fluid could be aspirated. If fewer than 4 quarter-hour specimens were obtained, because of rapid emptying of the stomach, a second (or third) experiment was performed by introducing another 200 cc. portion of the test-meal immediately after the last aspiration and continuing as before.

In all, 64 such experiments were performed with four different dogs. These experiments were divided into four groups, each characterized by a different type of test-meal; i.e., (1) 7 per cent alcohol, (2) distilled water, (3) isotonic NaCl solution (165 mN), and (4) hypertonic NaCl solution (330 mN). Each test-meal contained phenol red (40 mgm. per liter) as dilution indicator. Whereas alcohol was a suitable stimulus to gastric secretion, the water and saline test-meals usually provoked but little secretion, although in occasional experiments (particularly with dog A) the responses were found to be adequate. Since we were particularly concerned with actively secreting stomachs, histamine (in single subcutaneous doses of 0.3 or repeated doses of 0.15 mgm. per kilogram) was used as an auxiliary stimulus to these inadequate test-meals. Although the alcohol test-meal was an adequate stimulus in all cases, a few of these experiments were also supplemented with histamine, in order to be certain that this procedure introduced no gross difference in the results. The first dose of histamine was injected at the time the initial test-meal was introduced. Subsequent injections were given as the situation required.

Each sample of gastric contents was analyzed for free and total acidities, total chloride concentration, and phenol red concentration. The latter was determined by a method previously described (Hollander and Penner, 1940), the other chemical constituents by the standard procedures of this laboratory. The formulae for making the necessary calculations are presented in the Addendum. It should be noted that the equation for the corrected concentration of acid or chloride (C_s) automatically adjusts for the concentration of the acid or chloride initially present in the test-meal (C_t).

OBSERVATIONS. *Series I—Alcohol test-meal.* In the first series of experiments alcohol test-meals were used in order to confirm with dogs our previous observation on humans, concerning the occurrence of hypertonic values for *corrected* concentrations of chloride and acid. The results on a single such experiment are illustrated figure 1. The curves for *observed* concentration (broken line) are both reasonable approximations of the usual findings in fractional gastric analyses, with the highest value attained for acidity or chloride at 135 mN. In fact, throughout this investigation we never obtained a single *observed* concentration value above the isotonic range, 156–175 mN. In contradistinction to this, however, the curve for *corrected* chloride concentration (unbroken line) rises to a peak of more than twice the mean isotonic value of 165 mN, and only one of the

seven points constituting this curve falls below this critical value. No such obvious discrepancy is observable in the corresponding acidity curve, though other experiments of this series occasionally did show high values for this constituent.

In order to obtain some measure of the relative frequency of occurrence of these hypertonic values, the data for the entire series of experiments (14 in all) were analyzed statistically (table 1). From the frequency distribution for the chloride data, it appears that only 16 per cent of all the corrected concentration values fall within the isotonic range, whereas 62 per cent fall above it. In the case of the total acidity data, 8 per cent of

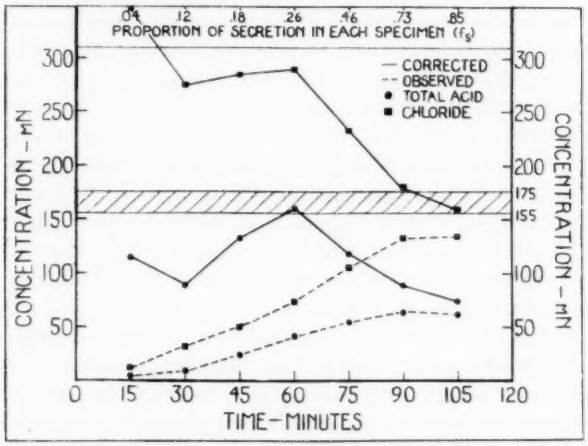

Fig. 1. Acidity and chloride curves with and without correction for dilution by the test-meal.

Experiment L-17. Dog B. Test-meal: vol. = 200 cc.; [alcohol] = 7 per cent; [phenol red] = 40 mgm./liter.

the values are within this range and 5 per cent above it. The frequencies of occurrence of unmistakably hypotonic values (i.e., less than 155 mN) are 22 per cent and 87 per cent for chloride and acid respectively. The difference between these two distributions is also well reflected by their means and their medians.

Series II—Water test-meal (supplemented with histamine). It was next necessary to determine whether the alcohol itself was specifically related to the occurrence of these hypertonic *corrected* concentration values. Hence a second group of experiments was performed with distilled water as the test-meal and histamine as the stimulus to secretion; figure 2 represents an illustrative experiment of this series. Here again the *uncorrected* concentration values appear to be normal whereas the corrected values show

TABLE 1

Frequency analysis of acid and chloride data corrected for test-meal dilution by the dilution indicator technique

(Observations on 4 unoperated dogs—A, B, C, and D)

SERIES	I		II		III		IV	
TYPE OF TEST-MEAL	Alcohol, 7 per cent		Water		NaCl, 165 mN		NaCl, 330 mN	
CONSTITUENT	Acid	Cl	Acid	Cl	Acid	Cl	Acid	Cl
Number (per cent) of specimens in each concentration range:								
155 mN or less	56 (87%)	14 (22%)	38 (62%)	8 (13%)	112 (97%)	39 (32%)	35 (100%)	22 (63%)
156–175 mN	5 (8%)	10 (16%)	13 (21%)	13 (21%)	3 (3%)	82 (66%)	0	13 (37%)
More than 175 mN	3 (5%)	40 (62%)	10 (17%)	40 (66%)	0	3‡ (2%)	0	0
Total*	64 (100%)	64 (100%)	61 (100%)	61 (100%)	115 (100%)	124 (100%)	35 (100%)	35 (100%)
Median	112	188	141	196	119	162	97	151
Mean	108	208	137	215	109	158	93	147
σ_{Mean}**	5.4	9.0	8.1	14.3	3.2	1.3	5.2	2.8

* Specimens with no free acid were excluded from this frequency analysis.

** This standard error is uncorrected for correlation; such correction would reduce its magnitude in each case.

‡ These three specimens possessed the following corrected chloride values: 177 mN (*55.5), 180 mN (*56.9), 179 mN (*57.5). (See Observations, Series III.)

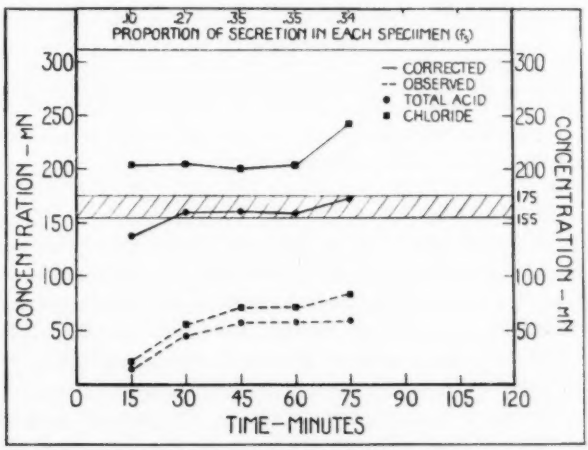

Fig. 2. Acidity and chloride curves with and without correction for dilution by the test-meal.

Experiment L-39a. Dog A. Test-meal: vol. = 200 cc.; water; histamine supplement; [phenol red] = 40 mgm./liter.

characteristics similar to those obtained with the alcohol test-meals. Every single chloride value in the solid line graph is greater than 165 mN, though none of the corrected acidity values of this particular experiment happen to exceed this value. A frequency analysis of the corrected chloride data for all 19 experiments in this group (table 1) reveals the following: 21 per cent of all the values fall within the range 165 ± 10 mN, with 13 per cent below and 66 per cent above this interval. Of the acidity values, 62 per cent are within the isotonic range, 21 per cent below it, and 17 per cent are distinctly hypertonic. These results including the means and medians, are in no way significantly different from those of series I, nor from those previously reported with human subjects.

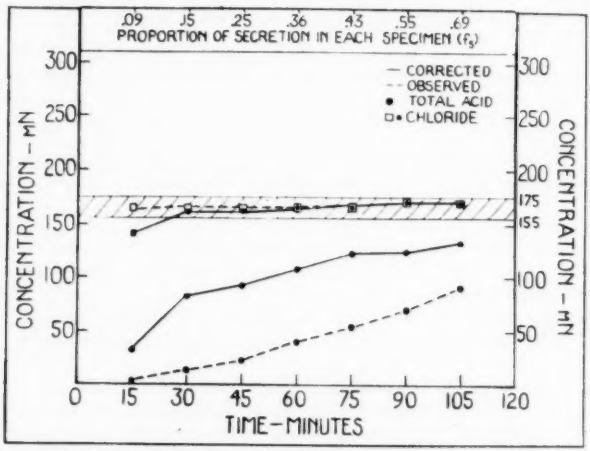

Fig. 3. Acidity and chloride curves with and without correction for dilution by the test-meal.

Experiment L-56a. Dog C. Test-meal: vol. = 200 cc.; [NaCl] = 165 mN; histamine supplement; [phenol red] = 40 mgm./liter.

Series III—Isotonic saline test-meal (supplemented with histamine). Since different hypotonic test-meals give essentially the same result with respect to the occurrence of hypertonic corrected concentrations, we next compared the data from these hypotonic test-meal experiments with those obtained by means of an isotonic test-meal. To this end a number of experiments were performed on the same animals with a test-meal containing 165 mN NaCl, supplemented with histamine injections. An example of this series is presented in figure 3. The curve for observed concentration (broken line) of total acid is grossly the same as in the previous series; that for observed chloride, however, is practically horizontal, indicating a constant concentration throughout the experiment. The cor-

rected chloride concentration graph likewise manifests a considerable degree of constancy, as can be seen from the corresponding (solid line) curve in the illustration. It is noteworthy that not a single corrected chloride value is significantly greater than 165 mN; actually, the highest value obtained in this experiment is 168 mN. The corrected acidity curve for this illustrative experiment likewise never attains a value greater than 165 mN.

The essential characteristics of this individual experiment, in contrast with those employing hypotonic test-meals, are borne out by a frequency analysis of the entire series of 34 experiments (table 1). For total acidity, not a single sample has a corrected value that is hypertonic, and less than 3 per cent of the specimens fall within the isotonic range. The corrected chloride data show only 2 per cent of such hypertonic values whereas the isotonic range contains 66 per cent. Actually, all three of the hypertonic values narrowly miss falling within the isotonic range, as well; their values are 177, 179 and 180 mN. This may possibly be the result of analytical errors only. However, it must be remembered that the constant, 165 mN, is only a statistical approximation to what is in effect an exceedingly variable physiological concept (i.e., isotonicity). Hence it is conceivable that the occurrence of even these few hypertonic corrected concentration values might have been obviated by a slightly different choice of test-meal concentration, relative to the osmotic equilibrium of each animal at the time of experimenting. It appears from these findings that the use of a test-meal containing 165 mN NaCl resulted in the practically complete elimination of hypertonic values for the corrected concentrations, in marked contradistinction to the results obtained with the hypotonic test-meals.

Series IV—Hypertonic saline test-meal (supplemented with histamine). If the isotonicity of the test-meal in series III is the sole or even the major reason for the absence of hypertonic corrected values, it may be expected that a hypertonic test-meal will be similarly effective in suppressing their occurrence. Accordingly, a few experiments were performed with a solution of 330 mN NaCl as test-meal; an illustrative example of these is given in figure 4. The two acidity curves (corrected and uncorrected) of this experiment are essentially the same as the corresponding curves of the experiment with isotonic test-meal illustrated in figure 3; they differ from the latter by no more than other experiments of the isotonic test-meal group differ from each other. The observed chloride curve, which shows a steady decline from 330 mN naturally differs from those previously presented because of the high initial chloride concentration in the test-meal itself. The graph for corrected chloride, however, rises with considerable regularity but never to a value above the isotonic range; the maximum for this experiment is 162 mN. Although the shape of this curve

is in no way typical of all the experiments in this series, the absence of hypertonic values is entirely characteristic. Thus, from the frequency analysis in table 1, it is evident that not a single corrected concentration value for either acidity or chloride is greater than 165 ± 10 mN. In fact, every single acidity value is below this range, in which respect this series resembles the isotonic test-meal group. As for the corrected chloride values, here also the range above 175 mN contains not a single value, whereas 37 per cent of them fall within the isotonic range and the remaining 63 per cent below it—in contradistinction to 66 per cent and 32 per cent for the corresponding values of series III. It is noteworthy that this differ-

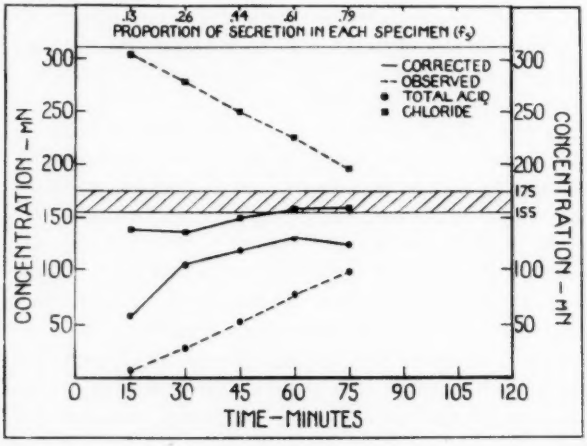

Fig. 4. Acidity and chloride curves with and without correction for dilution by the test-meal.

Experiment L-53. Dog C. Test-meal: vol. = 200 cc.; [NaCl] = 330 mN; histamine supplement; [phenol red] = 40 mgm./liter.

ence in frequency distribution for the chloride values of the two series of experiments is reflected both by their median and their mean concentration values. The medians are 151 and 162 for series IV and III respectively. The corresponding means are 147 and 158 and the difference between them (11) is equal to 3.7 times its standard deviation, without making allowance for correlation. Hence there can be no doubt that the mean chloride concentrations for these two groups of experiments are statistically different. A similar comparison of their acidity values supports this conclusion; the difference between the two means is 16, which is 2.6 times its own standard error. Whether this difference is due to absorption of solute, inhibition of acid secretion, or elaboration of a "dilution secretion," we cannot say at present.

DISCUSSION. It has been shown in the foregoing experiments that the use of a salt-free alcohol solution or of water alone (in conjunction with histamine) as test-meal in gastric analyses by the dilution indicator method frequently results in aberrant corrected concentration values. Taking the two groups of experiments with hypotonic test-meals[3] together (series I

TABLE 2

Frequency analysis of acid and chloride data corrected for test-meal dilution by the dilution indicator technique

(Observations on humans* and dogs with whole stomach pouches or unoperated stomachs**)

SERIES	Humans with unoperated stomachs*		Dogs with whole stomach pouches (group A)**		Dogs with unoperated stomachs (group B)**		Dogs (groups A and B combined)**	
TYPE OF TEST-MEAL	Alcohol, caffeine, water. (No chloride)¶		Liebig's extract. Total chloride 58-61 mN		Liebig's extract. Total chloride 57-62 mN		Liebig's extract. Total chloride about 60 mN	
CONSTITUENT	Acid	Cl	Acid	Cl.	Acid	Cl	Acid	Cl
Number (per cent) of specimens in each concentration range:								
155 mN or less	38 (83%)	16 (33%)	19 (47%)	2 (5%)	54 (95%)	23 (40%)	73 (75%)	25 (26%)
156–175 mN	4 (9%)	5 (10%)	6 (15%)	6 (15%)	3 (5%)	23 (40%)	9 (9%)	29 (30%)
More than 175 mN	4 (9%)	27 (56%)	15 (38%)	32 (80%)	0	11 (19%)	15 (16%)	43 (44%)
Total	46 (100%)	48 (100%)	40 (100%)	40 (100%)	57 (100%)	57 (100%)	97 (100%)	97 (100%)
Median	116	180	162	205	118	158	124	170
Mean	108	211	163	207	106	157	129	178
σ_{Mean}	6.5	18.8	7.5	5.9	4.1	2.9	4.8	3.9

* Data from study by Penner, Hollander and Post (1940).

** Data from study by Wilhelmj, O'Brien and Hill (1936).

and II), almost 65 per cent of all the Cl values and more than 10 per cent of the acidity values are greater than 175 mN. The incidence of such hypertonic values is equally striking in the studies of Wilhelmj and his co-workers (1936), who employed hypotonic Liebig's extract test-meals in dogs both with whole stomach pouches and with unoperated stomachs.

[3] Although 7 per cent alcohol is about 1.5 molar, there can be no doubt of its "hypotonicity" because of the ready absorption of alcohol by the stomach.

In their experiments, 44 per cent of the total chloride and 16 per cent of the acid chloride values are greater than 175 mN (table 2). Similarly, in our observations with salt-free test-meals on man, we found that 56 per cent of all the Cl values and almost 9 per cent of the acidity values fell above the isotonic range. On the other hand, when the test-meal in the present experiments consisted of isotonic or hypertonic salt solutions the incidence of such abnormal values was practically nil: less than 2 per cent for chloride and zero for acid concentration. A comparison of medians and means for these several groups of experiments supports this evidence. We may conclude, therefore, that there is a specific correlation between the presence or absence of such hypertonic values for corrected concentrations and the tonicity of the test-meal. Their lower incidence for acidity as compared with chloride can be ascribed to the neutralization process, which affects the former but not the latter.

In order to account for the occurrence of these hypertonic values, three possible hypotheses present themselves, based on the following: 1, the elaboration of a hypertonic secretion; 2, the summated effect of random analytical errors in the phenol red, chloride, and acidity determinations, and 3, the gastric absorption of water, which would increase the apparent concentrations of all solutes in the gastric fluid as determined by this method.

Of these three explanations, that based on a hypertonic secretion is least tenable, if only because such a secretion is unknown throughout the alimentary canal. The second explanation, based on the analytical errors of the method, merits more consideration. We have shown elsewhere (Hollander and Glickstein, 1940) that even though the standard errors of individual chemical analyses be suitably low, the mathematical manipulations of the dilution indicator method may combine these errors with considerable magnification. Thus, under certain conditions of test-meal concentration, C_t, and proportion of secretion in the gastric fraction, f_s, the standard error of a corrected concentration value, C_s, may be 50 per cent of that C_s-value, or even considerably greater. Under most conditions, and particularly when f_s is greater than 0.2[1], these errors remain within a limit, which, though large, is acceptable for our present purposes (i.e., a maximum standard error of 20 m.eq. per liter). Now, in order to determine whether the analytical errors alone are sufficient to account for the aberrant concentration values, we have studied the correlation of low and high f_s-values (i.e., less than and greater than 0.2) as compared with the incidence of hypertonic and non-hypertonic Cl-values in all our data. The results, arranged in two groups for series I and II and series III and IV

[1] An f_s-value of 0.2 means that the specimen of stomach contents contains two parts by volume (20 per cent) of secreted fluid and eight parts (80 per cent) of residual test-meal.

respectively, are summarized in table 3. The extremely low values for the coefficient of mean square contingency (0.06 for both groups of data) demonstrate the almost complete absence of correlation between tonicity of corrected Cl value and magnitude of f_s; i.e., the distribution of these gastric samples among the four cells falls just short of being entirely random. In other words, low f_s values (which are most likely to produce the greatest analytical errors) are associated no more frequently with hypertonic corrected concentration values than with non-hypertonic values. Hence, it may be concluded that the influence of analytical errors is but a minor one in this connection.

TABLE 3

Independence of tonicity of corrected chloride values and proportion of secretion (f_s)

	NUMBER OF f_s VALUES			COEFFICIENT OF MEAN SQUARE CONTINGENCY (PEARSON'S)
	<0.2	≥0.2	Totals	
Series I and II—Hypotonic test meals				
Number of Cl values:				
Hypertonic	26	54	80	$C = \sqrt{\frac{\chi^2}{N + \chi^2}}$
Non-hypertonic	12	33	45	$= 0.057$
Totals	38	87	125	
Series III and IV—Non-hypotonic test meals				
Number of Cl values:				
Hypertonic	1	2	3	$C = \sqrt{\frac{\chi^2}{N + \chi^2}}$
Non-hypertonic	32	124	156	$= 0.062$
Totals	33	126	159	

We are left, therefore, with the third hypothesis—that based on fluid absorption. This explanation, together with some supporting evidence, was advanced by Wilhelmj, O'Brien and Hill (1936), but these authors associated the absorptive process with variations in acidity rather than in osmotic concentration of the stomach contents. Although early workers, Edkins (1892) and Von Mering (1893), found no evidence for gastric absorption of water, Faitelberg (1930) and Sleeth and Van Liere (1937), by means of improved techniques, were able to prove that this phenomenon does occur. Hence, such an explanation for the occurrence of hypertonic corrected concentrations is clearly a tenable one. It may be argued that the rate of water absorption under our conditions of experimentation is likely to be very small, and quantitative evidence in support of this contention can be found in the several investigations just cited. However, by means of the mathematical relations developed previously (Hollander

and Glickstein, 1940) it can be shown that the influence of such a small water loss on the corrected concentration values may be very great, because the entire effect of this loss operates in diminishing the apparent volume of secretion in the gastric specimen but not at all the volume of residual test-meal. *In other words, the dilution indicator technique involves the tacit assumption that the test-meal retains its identity (original composition) throughout the experiment, no matter what changes occur in the stomach contents as a result of secretion and absorption.* Such a decrease in the apparent volume of secretion must yield an increase in the apparent concentration of all its solutes provided, of course, that these solutes do not undergo differential absorption. By way of illustrating this point without resort to a technical mathematical analysis, it has been shown in a purely hypothetical situation (Penner, Hollander and Post, 1940) that parietal secretion with a chloride concentration of 165 mN is capable of yielding an apparent corrected chloride concentration of 330 mN (i.e., an error of 100 per cent) solely as the result of the absorption of as little as 5 cc. of fluid during the period of observation.

Further evidence in support of the absorption hypothesis, however, is afforded by the fact that these excessively high values are essentially nonexistent in the two series of experiments employing isotonic and hypertonic test-meals. Whether such test-meals eliminate fluid absorption completely or merely reduce its speed to a very low rate is a moot question, but the simultaneous elimination of hypertonic corrected concentration values indicates a probable relation between these latter values and such absorption. Furthermore, Wilhelmj (1938) has found "a fairly good number of experiments on whole stomach pouches in which there was a rise in the . . . concentration of the phenol red to values of 114 and 120 per cent of the meal," which he accounted for by a differential absorption of water and concentration of the non-absorbable indicator. We have never observed this phenomenon in our own work, but in the course of several of our experiments (L-36, 39, and 46) we have noted that the usual progressive drop in phenol red concentration was followed by a secondary rise, suggestive of an absorption rate which exceeded the rate of secretion during the latter part of the experiment.

In view of the foregoing observations, there can be little doubt that 1, hypertonic corrected concentration values are wholly erroneous, and do not reflect the true composition of the secretion; 2, they are traceable chiefly to fluid absorption by the gastric mucosa and only in small part to the analytical errors of the dilution indicator technique; 3, where they occur as a result of fluid absorption, *all* the other corrected concentration values of the same experiment must also be considered erroneous, and 4, their occurrence can be prevented almost entirely by the use of an isotonic salt test-meal. The addition of secretagogue substances (as contained in

Liebig's extract) to such a test-meal ought not to affect the results any more than a subcutaneous injection of histamine given parallel with the test-meal. Alcohol, however, should not be added to the test-meal as a gastric stimulus in such experiments, because it may be absorbed at a much greater rate even than water. Another complicating factor which may be present is the absorption of acid and salt, in which case it will also contribute errors of indeterminate magnitude in the use of the dilution indicator method.

Fluid absorption in the course of experiments employing the dilution indicator technique may seem to be of minor physiological importance, because of the low absorption rate. Also, it may be contended that the same absorption process obtains in ordinary gastric analyses without consequential effect on the (uncorrected) acidity and chloride curves. The significance of the present investigation, however, lies not in the mere existence of such absorption but in the fact that *its occurrence will give rise to values calculated for the fraction of the test-meal, the fraction of secretion, and the corrected concentrations of the constituents of the secretion which are erroneous.* In consequence, any data except total amounts of solute which may be derived from these erroneous values (e.g., the volume of secretion contained in a gastric specimen or the concentration of chloride and base in the non-acid secretion) are likewise open to error. Since Wilhelmj and his co-workers have based many of their diverse studies on the dilution indicator technique with phenol red and Liebig's extract test-meals of low salt concentration, their observations are open to question on this score and must be repeated with isotonic salt test-meals before all their conclusions can be accepted.

SUMMARY AND CONCLUSIONS

1. Gastric analysis experiments, based on the dilution indicator technique with phenol red, were carried out on unoperated dogs. When the test-meal was alcohol, or water supplemented with histamine, it was found that many of the corrected concentration values for total acid and total chloride were hypertonic. Such abnormally high values had been observed previously by us in humans and by Wilhelmj and his co-workers in dogs. In all these instances, the test-meals were either salt-free or contained salt in less than isosmotic concentration.

2. When isotonic or hypertonic salt test-meals were used, no such abnormally high values were obtained, except for a very few which might be related to errors in the chemical analyses.

3. For this and other reasons, these high values are ascribed only in small part to the analytical errors of the dilution indicator technique, but chiefly to absorption of fluid by the gastric mucosa.

4. Hence, these hypertonic values are wholly erroneous and do not re-

flect the true composition of the secretion, as has heretofore been assumed. In any such experiment which yields hypertonic corrected concentration values not explainable by analytical errors, it must be presumed that fluid absorption has taken place, and *all* the corrected concentration values (even those *not* hypertonic) must be considered spurious.

5. We believe that the dilution indicator technique should be used only with isotonic test-meals. This will minimize the errors due to water absorption and may even eliminate them entirely.

6. Any experiments by the dilution indicator method, designed to yield *volume or concentration data* concerning the various constituents of the secretions of the stomach, are open to question if hypotonic test-meals have been used. Such investigations must be repeated with isotonic test-meals before all their conclusions can be accepted. Conclusions concerning *amounts* of the several components, however, are not subject to this error unless the individual components undergo absorption.

ADDENUM. The following formulae are those used in the dilution indicator method of gastric analysis; for their mathematical development see the previous report by Hollander and Glickstein (1940).

$$f_t = \frac{P_0}{P_t}$$

$$f_s = \frac{P_t - P_0}{P_t}$$

$$C_s = \frac{C_0 - C_t f_t}{f_s} \quad \text{or} \quad C_t + \frac{C_0 - C_t}{f_s}$$

where C_s = the concentration of any component, as acid or chloride, in the mixed secretion; i.e., the *corrected concentration* value.

C_t = the concentration of this same component in the test-meal.

C_o = the concentration of this same component in any gastric specimen, i.e., the *observed concentration* value.

P_t = the concentration of phenol red in the original test-meal.

P_o = the concentration of phenol red in the gastric specimen.

f_t and f_s = the proportions by volume of test-meal and mixed secretion in the gastric sample; i.e., $f_t + f_s = 1$.

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INTERACTION OF MEDULLATED FIBERS OF A NERVE TESTED WITH ELECTRIC SHOCKS

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In 1932 Blair and Erlanger described experiments performed with the object of ascertaining whether activity of some of the medullated fibers of a nerve trunk *increases* the excitability of inactive medullated fibers in the same trunk. The results in *normal* nerve were negative. Since then, Jasper and Monnier (1938) have demonstrated the transmission of the impulse from one nerve to another in juxtaposed preparations of crustacean (nonmedullated) nerve, but only when the receiving nerve is on the verge of spontaneous discharge.

More recently Katz and Schmitt (1939, 1940), employing two adjacent giant fibers of *Carcinus*, have demonstrated a triphasic effect on the excitability of one fiber by a propagated disturbance in the other, consisting in succession of 1, a fall (beginning before the arrival of the spike); 2, a rise, each amounting to about 20 per cent of the threshold, followed by 3, a much lower and longer fall. They attribute this excitability sequence to the current emanating from the active locus which reverses twice as the latter progresses.

Under the circumstances we have reinvestigated the interaction of medullated fibers employing this time a method that would disclose not only increased response, which was all our previous method could disclose, but decreased response also. The experiments have disclosed that there is induced an early increase in response which in normal nerve, however, is completely masked through shunting of the testing shock by the conditioning fibers, whose resistance is low while they are in a state of activity.

METHODS. In principle the method consists of starting a maximal spike, the conditioning action potential, along one dichotomous branch of a large mixed nerve. At appropriate intervals thereafter a submaximal stimulus is applied to the parent nerve and the resulting response is recorded from the other branch. The change in the height of the response as the testing stimulus falls, in successive trials, in every relation to the conditioning action potential expresses the effectiveness of the testing stimulus as modified by the conditions of the experiment.

The sciatic nerve of the green frog (*Rana pipiens*), with attached peroneal and tibial branches, was the most frequently used preparation. The preparations were usually equilibrated in Ringer's solution for one or more days at 5°C. and during experiments were mounted in a moist chamber, in air, at room temperature.

Electrode arrangements. The preparation (see figs. 1 and 2) usually was suspended by the two branches containing the conditioning and the conditioned fibers, which were held apart by silver electrodes. A large silver plate (13 x 25 mm.) was placed against the nerve roughly centered on the tibial-peroneal bifurcation. In some experiments the plate was placed horizontally so that it would, by capillarity, hold on its upper surface a pool of Ringer's solution into which the part of the nerve on the plate could be immersed. The plate was grounded and acted as the cathode of the conditioning stimulus applied to the tibial, as the cathode of the testing stimulus to the sciatic and, interchangeably, as one amplifier lead (ground) for the recording of the conditioning spike in the sciatic nerve or of the conditioned spike in the peroneal nerve. Other silver electrodes appropriately placed, completed the above circuits. This simple electrode arrangement so completely eliminated shock escapes that it usually was necessary to connect an aerial to the amplifier input in order to introduce a shock artifact when a record of the instant of stimulation was needed. Since there is only one ground connection to the nerve, interaction is minimized of the various circuits through ground surges, such as occurred in the earlier experiments in which multiple electrodes took the place of the plate. Under the earlier conditions interaction of the stimulating circuits sometimes occurred, but such interaction involved no conduction delay and could easily be identified and distinguished from the effect of the conditioning response, which appeared only after a significant conduction time.

Recording. The activity of the nerve has been recorded by leading the action potentials through the electrodes mentioned above into an amplifier which drives an electron oscillograph. Changes in the response of the fibers at the tested locus in the sciatic express themselves as changes in the height of a submaximal spike initiated there and recorded from the conditioned fibers in the peroneal. In practice the maximum conditioned spike from the peroneal has been brought to a selected amplitude on the oscillograph by varying the amplification. Then the change in height with the change in voltage of the testing shock is observed throughout the submaximal range and the shock strength fixed at such a level that small changes in the stimulating voltage produce maximum changes in spike height. Usually the strength selected has produced a spike which is somewhat less than half maximal in height. This fixed shock is applied to the tested locus of the sciatic at variable intervals after a maximum

conditioning action potential is initiated in the conditioning nerve, and the effect of this action potential on the peroneal fibers as conditioned by it at the sciatic locus manifests itself as changes in height of the action potential initiated there and recorded from the peroneal nerve. In some of the later experiments the deep and the superficial tibial nerves were employed as the conditioning and the conditioned branches, respectively.

As many responses to the testing stimuli as could be distinguished from each other, scattered over the temporal range under observation, have been photographed as multiple exposures on a single film (see figs. 2 and 3), and from seven to ten of these films have been used in the construction of each of the response curves. Chance variations in excitability have thus been reduced to a minimum.

Stimulation is accomplished by short shocks (RC $\simeq 1 \times 10^{-4}$ sec.) from independent gas discharge tube stimulators, delivered to the nerve through low capacity, shielded transformers. The cathode ray sweep circuit and the two stimulators are activated through delay circuits by a master oscillator usually discharging at the rate of about 70 per minute.[1]

Results. At first the response curves derived from different points along a preparation varied quite unaccountably, though the same point continued to yield its original record. Eventually, when the conditions determining the variability came to be understood, reasonably constant and predictable pictures could be obtained. Most of the disturbing variations in the response curve were found to be referable to polarization of the tested locus by action and injury currents acting through stray circuits permitted by branches, cut and uncut, or by some other discontinuity in the nerve's structure.[2] A potent source of distortion, for example, has been the tibial-peroneal junction. It was not realized, at first, that the junction which presumably determined the distortion is not, or not to any degree, the crotch between the two nerves, but lies rather somewhere proximal[3] to this point, probably where the connective tissue septum ceases to be an effective electrical barrier between the two sets of nerve fibers. Between that point and the ground there will be an additional path through which could flow a part of the current determined by the potential drop between the confluence and the grounded lead. During the period of such current flow, which would be coincident with the passage of a spike, the excitability of the fibers at the tested locus would be altered, —elevated if the flow polarized the tested locus cathodally, depressed if it were polarized anodally. In order to minimize this particular action

[1] For further details concerning method consult the legends to the several figures.

[2] For a discussion of the conditions that may distort action potentials as led from the nerve's surface consult Bishop, Erlanger and Gasser (1926).

[3] Terms of direction refer to the normal anatomical relations of the sciatic nerve, not to the direction of impulse transmission.

the preparations eventually were mounted with the tibial-peroneal crotch on the grounded plate as far from the testing cathode as was consistent with securing a clean, unbranched stretch of sciatic of sufficient length beyond (central to) the grounded lead. Generally the crotch has occupied a position about 10 mm. distal to the tested locus; and then there was left a clean stretch of sciatic something over 10 mm. in length between the tested locus and the first of the larger thigh branches. This distance proved to be just about sufficient to reduce to insignificance the disturbing

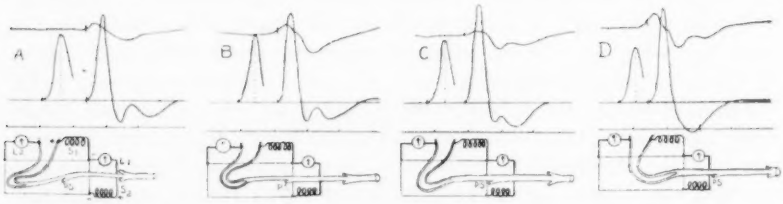

Fig. 1. Illustrating some of the variations in the configuration of the response curve referable to structural and injury factors.

The position of the proximal portion of the sciatic nerve was changed for each trial as indicated in the diagrams. The distance between the terminals of S_2 was 7.4 mm. PS = peroneal stump.

Differences in the configuration of the response curves as the nerve is moved are quite obvious. It would not be profitable to account specifically for them.

The following applies to all figures. From above downwards are shown the response curve, the relation to it of the conditioning spike (the second in each case), the time in msec., and a diagram of the circuit in relation to the preparation as used. The nerve length is drawn to scale. S_1 = conditioning stimulator; L_1 = lead for recording conditioning spikes; S_2 = testing stimulator; L_2 = lead for recording the conditioned responses. The grounded lead is a silver plate outlined by lighter lines. Wires are indicated by the heavier lines. The crest of the first spike marks the arrival of the conditioned spike at L_2. The second is the conditioning spike recorded at the conditioned locus through L_1. In the diagrams it is set with the escape of the shock initiating it on the crest (extended to the base line) of the first spike; the beginning of the second spike (indicated by the mark on the response curve) therefore marks the time conditioning begins at the tested locus.

effects of injuries, branches, etc. in the proximal region on excitability at the tested locus. Some notion of the complications encountered when precautions are not taken to work under conditions that minimize these sources of distortion may be obtained through inspection of the response curves (fig. 1) derived from one of the experiments performed with a view to ascertaining sources of distortion.

When every indicated precaution is taken to avoid these disturbing factors the response curves have the configuration seen in figures 2, A and B, and 4, A. At the instant of the arrival of the conditioning spike at the tested locus the response elicited from the test fibers diminishes

and it then remains low for something less than the duration of the spike, when the latter is recorded monophasically. In the trough of curves of this type, with rare exceptions, an upward bowing can be detected beginning before the conditioning spike attains its crest. The response then grows, rapidly at first, along a curve which becomes convex upwards and passes above the normal level before it begins to bend toward it. Since the periods of observation succeeding the arrival of the spike at the tested locus have not exceeded about 4 msec. nothing is known regarding the final stages of the response cycle.

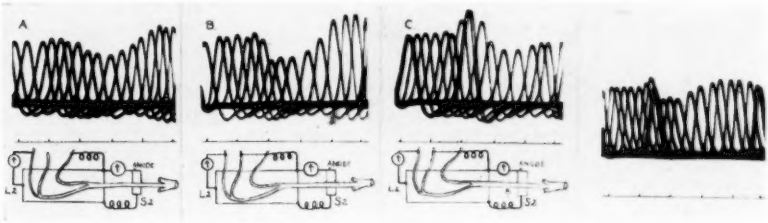

Fig. 2

Fig. 3

Fig. 2. Illustrative records of response changes, reproduced in approximately the photographed size. The crests of the spikes outline the response curve, and 7 to 10 such records, enlarged 4 times, are employed in the construction of each of the curves such as are copied in the other figures.

A, conditioning through the peroneal; B, conditioning through both the peroneal and the deep tibial; C, same as B, but after killing the nerve at the point indicated by the arrow, 5.8 mm. from conditioned locus. The conditioned fibers conduct into the superficial tibial in all cases. The cathode-anode distance of S_2 is 8.7 mm.

Fig. 3. A record from another preparation showing a slightly different type of response curve. Superficial tibial conditioned via deep tibial. An initial increase in response precedes the arrival of the spike at the conditioning locus, possibly an effect produced by the tibial-peroneal junction resting on the plate.

Some of the more frequently observed departures from this typical response may now be considered. Quite commonly the response begins to grow 0.2 to 0.4 msec. before the conditioning spike arrives at the tested locus (fig. 3). This rise usually can be attributed to the discontinuity at the tibial-peroneal confluence. If 30 m.p.s. be taken as the conduction rate, it would appear that a potential fall developing at such a discontinuity can affect the tested locus when the two are separated as widely as 6 mm. A much rarer departure from the rule has been a decrease in response beginning slightly before the conditioning spike leaves the grounded lead. Under the latter circumstances the arrival of the spike at the tested locus starts the characteristic, the more rapid, decline seen in the typical response curves. When the true tibial-peroneal confluence happens to be distal to the tested locus, but not too distant from it, the depressed portion of

the response curve may exhibit one or two prominent elevations which, however, rarely rise to the basal level. We were unable to account for these elevations before it became apparent that the true confluence is situated central to the tibial-peroneal crotch.

Crushing the nerve central to the tested locus, and within a range of about 10 to 12 mm., alters the configuration of the response curve. In figure 4 it is seen that the curve is unaltered when the nerve is killed 17.0 mm. proximal to the tested locus (B), and that killing at a distance of 6.6 mm. (C) completely changes it. A similar alteration, but of greater

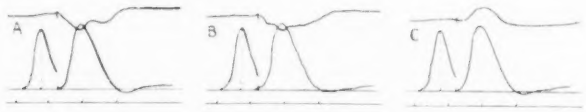

Fig. 4. A set of typical response curves.

A, separation of S_2 electrodes 19.0 mm.; B, the same but after killing at 17.0 mm.; C, separation of S_2 electrodes 8.1 mm.; killed at 6.6 mm.

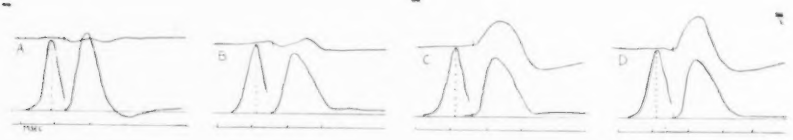

Fig. 5. Illustrating the effect of killing at different distances central to the conditioned locus.

The interelectrode distance for S_2 is 11.1 mm. A, before killing. This is the smallest response ever obtained from the frog's sciatic. The up and down effects must almost exactly have balanced. B, after killing at 8.5 mm.; C, at 5.6 mm.; and D, at 1.3 mm. distal to the tested locus. D is one of the rare instances in which, in a killed preparation, there is an initial "down" effect beginning *before* the spike arrives at the tested locus.

ultimate magnitude, is seen in figure 5. Here the change is slight when the distance between the injury and the lead is 8.5 mm. (B); it is much greater when the nerve is killed at 5.6 mm. (C); and greater still, but only slightly, at 1.3 mm. (D). The alteration in configuration consists of the replacement of the period of reduced response (practically absent in fig. 5, A) with one of increased response, and of the conversion of the upward convexity of the ending of the curve into an upward concavity approaching the normal level from below as the observation terminates.

We have next to account for the configurations of these response curves. In the first place it should be stated that when every precaution is observed to avoid complicating circumstances moving the anode of the testing circuit is without effect on their configuration. All of the processes that

change the configuration apparently must act, either directly or indirectly, through the locus under the cathode. Here it might be added that currents referable to action potentials or demarcation potentials acting through stimulating circuits have, in our experience, been without appreciable effect.

The trough in the curves derived from *uninjured* nerve must, we feel, be attributed to shunting of the stimulating current lines by a lowering of the resistance of the conditioning fibers with their entrance into activity. Since experiments we have done show that the depth of this part of the curve does not increase in proportion to the length of nerve subtended by the stimulating electrodes (the changes in length were made in short steps) it would seem to follow that changes in transverse resistance are in the main responsible for this effect.

Little is known regarding the configuration of the resistance changes associated with activity under the conditions of our experiments. Cole and Curtis[4] have shown (1939) that in single fibers the resistance decreases rapidly *after* the start of the spike and then increases more gradually toward the normal level. With the temporal dispersion that obtains in conducted multifiber spikes the decrease in resistance would be less steep than the rise of the recorded spike and less steep than in single fibers. We will assume, in the absence of specific information, that the resistance curve follows in general the configuration of the excitability cycle of nerve that has responded to a stimulus.

Now our "normal" nerves have recovered from refractoriness through a period of supernormality which has reached its maximum in about the same time as the response curves derived from normal preparations pass through their maximum during recovery. When the tested locus is under the influence of a demarcation current it is cathodally polarized, and it is known that cathodally polarized nerve in recovering from refractoriness does not pass through a supernormal period. Under these circumstances the resistance would be expected to return to the normal level without passing through a supernormal phase. On this basis it becomes possible to refer not only the earlier of the more prominent features of our "normal" response curves, but also the later, to variations in the shunting of the stimulating current used for testing, by resistance changes in the conditioning fibers.

It is possible, however, to account for the last phase of the response curve perhaps equally well on the basis of what is known regarding the influence exerted by polarization on the excitation cycle initiated by a *subthreshold* stimulus. If the conditioning spikes in the present experiments act as subthreshold stimuli to the tested fibers then (Blair)[5] when the latter are

[4] A bibliography of the subject is given by these authors.

[5] Cf. Erlanger (1939).

"normal" the excitability of the fibers would not at any time in the resulting cycle fall below the normal level. If, however, the tested locus were cathodally polarized, as by a demarcation current, the excitability following the period of latent addition would fall below the normal level and would remain subnormal for at least 3 msec. It is obvious then that through either set of assumptions the state of polarization of the tested locus could determine the configuration of the terminal phase of our curves.

But if the conditioning spike acts as a subthreshold stimulus the first response to it should be an increase in the excitability of the fibers it conditions. In *normal* nerve, however, the arrival of the action potential at the tested locus coincides with a decrease in response and it therefore becomes necessary to conclude that if there be any excitability increase due to latent addition it ordinarily is concealed by a simultaneous decrease in the effectiveness of the testing stimulus.

There is another consideration, besides those already mentioned, that suggests the inference that at least those parts of the response curve that coincide with the spike of the monophasic action potential are the resultant of two oppositely directed influences. It has been seen that practically invariably that portion of the curve gives evidence of an upward bulge with peak a bit later than the crest of the monophasic spike (see fig. 4, A).

This might be expected if this part of the curve were determined by the addition of two curves oppositely directed, one downward, having somewhat the shape of the curve of increased electric conductance (Cole and Curtis, 1939), the other of an upright monophasic spike lower in amplitude than the former. If the crest of the spike appeared while the resistance is decreasing rapidly, it would be displaced to the right with respect to the spike crest, just as is the upward bulge in our curves.

But perhaps the best evidence of the presence of an underlying excitation effect in the "normal" nerve is supplied by the gradual transformation of a "normal" response curve beginning with apparent depression, into one in which enhancement replaces the depression, by bringing a killed region closer and closer (within limits) to the tested locus (see fig. 5). This transformation very largely is referable to the increase in excitability determined at the tested locus by cathodal polarization. Since excitation by subthreshold stimuli is a function of the strength of the stimulus, it must be supposed that the action potentials that produce no obvious upward bulge in the "normal" response curve are nevertheless exerting their proportional effects in that direction.

Some conception of the magnitude of the changes in stimulus effectiveness that are determined by a passing spike can be obtained by comparing the variation in the height of the conditioned spikes elicited by a constant testing stimulus, with the variation in the height of the spikes in the same fiber group elicited by stimulating with shocks of known strength. It

is scarcely necessary to add that the values obtained will vary with changing conditions; that they will be qualified by the number of the conditioning fibers, the intimacy of their mixture with the fibers conditioned, the height of the composite spike as determined by the degree to which the axon spikes are in phase, and so on.

In normal nerve, then, we are dealing with a degree of excitation by the action current that is concealed by shunting of the testing current. The combined result is depression and this has been the equivalent of a reduction in shock strength of over 10 per cent.

The increase in excitability becomes apparent only when the tested locus is under the influence of a demarcation current. The largest increase we have seen has amounted to an equivalent increase of 26 per cent in shock strength. Since this increase is based on the depression produced by the shunting action of the conditioning fibers, the total increase in excitability must be equal to something more than the sum of the two values given above, or to an effective change of over 36 per cent of the shock strength.

Discussion. The curves derived by us from *normal* nerve show no resemblance to those constructed by Katz and Schmitt. On the other hand, from loci under the influence of a demarcation current, and therefore highly excitable, pictures have been obtained, though exceedingly rarely, that match exactly, in sign and in temporal correlations, those derived by them through a study of the action of one giant fiber upon another. The closest match is seen in figure 5, B. Here, as in Katz and Schmitt's curves, this curve begins in a direction indicative of a decrease in excitability that starts before the conditioning spike arrives at the tested locus. As in all other of our curves obtained under these conditions, this one then conforms with those obtained in experiments on giant fibers. Now it may be relevant to note that in this particular case (see fig. 5, A) the changes in stimulus effectiveness exhibited by the normal nerve were unusually slight. This situation might signify that in this particular instance the excitability changes were so great that they almost completely masked the shunting action, and therefore that the pictures obtained when the excitability of the tested locus was increased by the demarcation current were qualified still less by the resistance factor. If these actually were the conditions, it would be necessary to conclude that the sequence of the excitability changes induced by the action of a spike in one fiber on a contiguous fiber is the same in both medullated and nonmedullated fibers when resistance changes associated with activity of the conditioning fiber, when medullated, are discounted. We have seen, however, that there are other factors involved in the production of these response curves than the excitability changes determined by current flow from the active fiber. What the relative significance of all of the factors may be remains to be determined. It is quite possible that resistance changes may be

found to be much less significant in nonmedullated than in medullated fibers.

Observations on the phrenic nerve. A few similar observations have been made with the dog's phrenic nerve, desheathed, employing the same general technique. The conditioning maximal stimuli were applied to one of the trunks of origin of the nerve, the testing submaximal stimuli to the nerve well beyond the union of the trunks, and the variations in the height of the spike elicited by the latter stimuli were followed in the other trunk of origin. In previous work, employing a method which could indicate only an increase in excitability or an increase in stimulus effectiveness, the conclusion was reached that a passing spike does not *increase* the excitability of adjacent fibers, though an increase was demonstrable after supplying conditions that might have caused a demarcation current to flow through the tested locus (Blair and Erlanger, 1932).

The present experiments on the phrenic nerve confirm the earlier ones and show that, as in frog's nerve, a passing spike actually lowers the response. The pictures, though, have changed unaccountably with shifting of the anode of the testing circuit. Based on our experience with frog's sciatic, it would seem reasonable to assume that the desheathed phrenic is not as uniform a structure as it appears to be,—that the variability of the pictures with change in electrode position is referable to local injuries and discontinuities resulting from the removal of the sheath. The difficulty could not have been avoided by leaving the sheath on, since the surface of the sheath also is ragged. The amplitude of the response changes in the phrenic has been very much lower than in frog's nerve. The reason for this has not been investigated.

Functional significance. That the lowering of stimulus effectiveness seen in these experiments could be of any physiological significance in the normal functioning of nerves seems highly improbable.

In the central nervous system, however, the situation might be different. The final ramifications of fibers in the nerve centers are so closely packed that interaction could readily occur between them. Assuming that synaptic transmission can be accomplished electrically, it is conceivable that such transmission would be aided by poor electrical conductivity of surrounding tissues (which would be their state during inactivity) and hindered or, may we say, inhibited, by good electrical conductivity (during activity of surrounding tissues). Since in the central nervous system summation of impulses usually is required for synaptic transmission it is obvious that even a very slight variation in shunting of current lines might make the difference between transmission and failure of transmission.

In this connection attention may again be called to the enormous increase in the efficacy of issuing current lines as stimuli that results through cathodal polarization (by demarcation currents) of the fibers they act on.

Though the fact is as old as the rheoscopic nerve-muscle preparation, this demonstration of its relation to from-fiber-to-fiber action in a nerve trunk reminds us of the important rôle varying degrees of polarization could play in the functioning of the central nervous system.

SUMMARY

A method is described of ascertaining graphically the changes in the effectiveness of a stimulating current on inactive medullated fibers of a nerve trunk when other medullated fibers of the trunk are conveying impulses.

In "normal" nerve (frog's sciatic) as the multifiber spike passes by the tested locus the main features exhibited by the curves are a depression, about coincident with the monophasic conditioning spike, followed by an elevation preceding the return to normal.

In nerve "killed" not further than 10 to 12 mm. from the tested locus the depression is replaced, to a degree depending on proximity to the injured locus, by an elevation and this is followed by depression. In recovering, the latter does not pass above the normal (resting) level during the period of observation.

This development of a spike in the response curve is attributed primarily to an increase in excitability induced by the demarcation current; and it is inferred that the absence of a "spike" in the "normal" curve is the result of masking effected by a concurrent reduction in the resistance of the conditioning fibers, resulting in the shunting of the testing current.

The possibilities are considered *a*, that resistance changes might be responsible also for other features of the response curves, and *b*, that latent addition might enter as a factor.

It is suggested that the "resistance cycle" might play a rôle in synaptic transmission in the central nervous system.

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THE RÔLE OF LACTIC ACID IN THE MOVEMENTS OF POTASSIUM

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A large number of conditions are now known in which potassium enters the blood from the tissues. Some of these have been reviewed recently by one of us (Fenn, 1940), and it was pointed out that frequently lactic acid appears in the blood at the same time as potassium. The possibility was suggested, therefore, that the potassium enters the blood in the form of potassium lactate. In this paper it is proposed to present a number of recent experiments which bear upon this question. They tend to show that there is no necessary connection between the lactic acid and the potassium.

METHODS. Potassium in the plasma was analyzed by Wilde's (1939) modification of the Shohl and Bennett method and lactic acid in whole blood by Koenemann's (1940) modification of the method of Miller and Muntz. The pH was measured with a Beckman pH meter using a glass electrode of the type described by Pickford (1937) which permits samples to be withdrawn from under a layer of oil or from a tonometer after equilibration with 5 per cent CO_2 and measured without exposure to air.

Cats were anesthetized with dial. Other details are described in what follows or by Fenn, Wilde, Boak and Koenemann (1939) or Fenn, Koenemann and Sheridan (1940).

RESULTS. *Asphyxia.* As shown by Civin and Cattell (1938) and others there is a rise in plasma potassium when a cat is asphyxiated by clamping the trachea. A similar experiment on a cat with additional information concerning the pH of the arterial blood and its lactic acid concentration is plotted in figure 1. Four successive periods of asphyxia produced in this way are shown. In each case there was a sharp rise of potassium accompanied by a smaller rise of lactic acid.[1] At most, therefore, only a part of

[1] The smaller rise of lactic acid compared to potassium might possibly be due to a more rapid removal from the blood by inactive tissues. To test this point in one experiment we injected 5 cc. of 0.19M potassium lactate intravenously into a cat and analyzed the plasma at intervals thereafter for both K and lactate. There was some indication 8 or more minutes after injection of a more rapid decrease of lactic

the potassium increase could be associated with lactic acid. At the same time the pH of the arterial blood became more acid while the pH of the

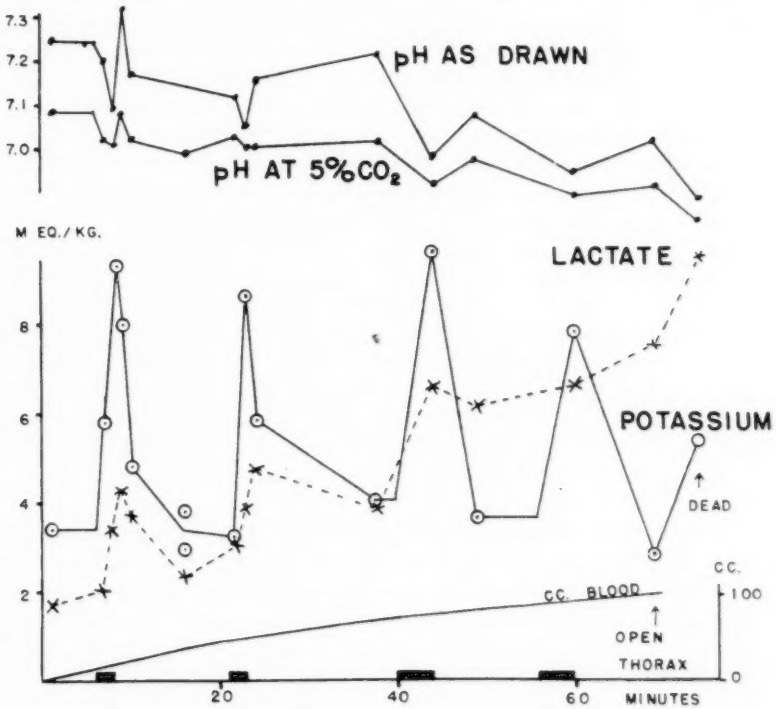


Fig. 1. Effects of four periods of asphyxia produced by clamping the trachea. Two periods lasting two minutes and two at four minutes. Artificial respiration was given at the end of each period until spontaneous breathing was resumed. Cat weight, 3.4 kgm., male. Received 2.2 cc. of dial intraperitoneally. Hematocrit dropped from 35 per cent at the beginning to 23 per cent at the end. Lactic acid refers to whole blood and potassium to plasma. Last sample from the heart; all others from the carotid artery.

acid than of K probably because it can disappear by metabolism as well as diffusion. The potassium never returned to the pre-injection level and indeed it showed a secondary rise after 20 minutes as described by Wilde (1939) while lactic acid finally went lower than its original level. Two minutes after injection, however, the increase in lactic acid calculated for plasma was actually 10 per cent greater than the increase in K and the volumes of distribution of K and lactic acid respectively were only 11.2 and 10.4 per cent of the body weight. In this time, therefore, only small but nearly equal amounts of K and lactate had left the plasma. The increases in concentration in figure 1 occur within a period of the order of 2 minutes and the K and lactate curves run parallel.

same blood after equilibration with 5 per cent CO_2 and 95 per cent oxygen showed smaller or irregular changes usually in the direction of increased acidity. We have records of another experiment identical with that shown in figure 1 in all essential particulars. It would not appear from these experiments that the K in excess of lactic acid could have entered the blood as KOH without any other changes.

It is possible that the potassium came into the blood in order to compensate for the increase in fixed acid resulting from the anaerobic metabolism, but the evidence for such an effect is not impressive. Somogyi (1940) reports an insignificant rise of K after injection of sodium lactate. In one cat we injected 6 cc. of 0.1 M HCl into the artery and found no change in plasma potassium 2 minutes later and only an insignificant increase after 6 minutes just before the death of the cat. In perfusing the legs of frogs lactic acid (half neutralized with NaOH) was added to the perfusate without causing a perceptible increase in the venous potassium. (Before lactic acid (0.005N) 3.34 and 3.81 m.eq. of K per liter; after lactic acid 3.66, 3.62, 4.51 and 3.97 m.eq. of K per liter in successive 2–6 minute periods.) Moreover, according to earlier experiments an increase of an easily penetrating acid like carbonic acid or lactic acid would be expected to cause a migration of potassium in the reverse direction, i.e., from the plasma to the tissues (Fenn and Cobb, 1935).

It is not likely that the reverse is true, i.e., that the lactic acid increased in the blood as a response to the increase in potassium because in two frog perfusion experiments we have injected KCl without finding significant changes in the lactic acid concentration of the blood. It is probable, therefore, that the potassium comes out in asphyxia as a consequence of the sympathetic stimulation and the action of adrenalin and that the lactic acid is the sign of a shift from an aerobic to an anaerobic type of metabolism.

Hemorrhage. In the experiment of figure 1 it can be seen that the lactic acid level steadily increases during the experiment. This may be taken to indicate incomplete recovery from the asphyxial periods; it may also be the result of the hemorrhage which was of considerable magnitude as indicated by the lower graph showing the amounts of blood withdrawn during the experiment. To study further the importance of hemorrhage in this effect some experiments were tried in which cats were subjected to acute hemorrhage by the withdrawal of successive large blood samples for analysis. A typical experiment of this sort is shown in figure 2. Both lactic acid and potassium increase during the bleeding and the pH of the arterial blood as drawn under oil and also after equilibration with 5 per cent CO_2 and 95 per cent oxygen showed steadily decreasing values. An interesting and characteristic variation from this tendency to acidity is seen in the temporary increase in alkalinity of the arterial blood after the

fourth blood sample. This is probably due to the over ventilation observed during the experiment because it does not appear at constant carbon dioxide tension.

Table 1 shows figures obtained in three cats subjected to hemorrhages of this sort. The last three columns give the changes in plasma potassium, whole blood lactic acid, and arterial pH after equilibration at 5 per cent CO_2 and as drawn under oil. Since the change in the concentration of

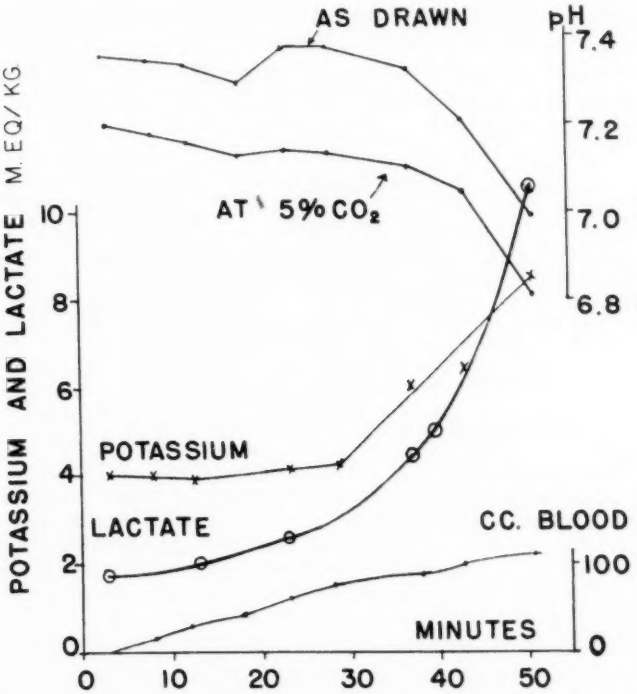


Fig. 2. Effect of hemorrhage in a cat, male, 2.9 kgm. after 1.9 cc. of dial. Last blood sample taken from the heart; others from the carotid artery.

lactic acid in the plasma would be even larger (about 20 per cent) than that in whole blood, it is evident that the number of equivalents of potassium which entered the blood is far less than the number of lactic acid equivalents. It is possible, therefore, that all the potassium came out as lactate but that not sufficient potassium could be mobilized to neutralize all the lactic acid. The data do not prove, however, any necessary connection between the two substances.

It was shown by Johnston and Wilson (1929) that in hemorrhage the

decrease in the alkali reserve was greater than could be accounted for by lactic acid alone. The simultaneous increase in potassium was not taken into consideration by these authors and would make it still more difficult to account for the decrease in alkali reserve. To one sample of cat's blood we added varying known amounts of lactic acid at a constant CO_2 tension of 5 per cent and measured the resulting pH. We found that an addition of 10.3 m.eq. of lactic acid per liter of blood, the average increase observed in the experiments of table 1, caused a decrease of pH of 0.34 from 7.40 to 7.06. While exact comparison with the Δ pH observed in the experiments of table 1 is not strictly justified because of variations in buffering of different bloods, nevertheless it is striking that this figure is so close to the Δ pH at 5 per cent CO_2 actually observed, i.e., 0.36. On this

TABLE 1

Electrolyte changes due to hemorrhage

NUMBER	WEIGHT OF CAT	BLOOD	TIME BETWEEN SAMPLES	INITIAL SAMPLE				CHANGE DUE TO HEMORRHAGE			
				Arterial pH		K	Lactate	Arterial pH		K	Lactate
				as drawn	at 5% CO_2			as drawn	at 5% CO_2		
	kgm.	*cc.*	*min.*			*m.eq./l.*	*m.eq./l.*			*m.eq./l.*	*m.eq./l.*
1	3.4	156	53	7.40	7.25	3.92	1.32	−0.18	−0.52	+3.39	+11.43
2	3.8	98	28	7.72	7.37	4.80	1.63	−0.04	−0.18	+2.70	+10.70
3	2.9	123	54	7.36	7.20	4.09	1.82	−0.37	−0.38	+4.51	+8.78
Average									−0.36	+3.53	+10.30

Cats anesthetized with dial. Samples taken at intervals from carotid artery. Data from first and last samples only are given. After the last sample recorded in the table second cat gave 70 more cc. of blood before death 24 minutes later. The Δ pH at the end was −0.62 as drawn and −0.46 at 5 per cent CO_2. Potassium is calculated per liter of plasma and lactic acid per liter of whole blood.

basis the change in pH is explained by the lactic acid and the change in potassium must be ascribed to something else, perhaps an exchange with sodium.

Adrenalin. The results of six injections of adrenalin in three cats are shown in table 2. Blood samples were taken a few minutes before and one minute after adrenalin in each case. As would be expected from the literature (d'Silva, 1934 and others) there was a marked increase in plasma potassium in each case which is on the average 24 times as great as the small increase in lactic acid which appeared in at least two of the cats. The increase in lactic acid is of the same order of magnitude as that reported by Griffith, Lockwood and Emery (1939). At the same time there was in all cases an increase in corpuscular volume probably due to contraction of the spleen, and a decrease in both Na and Cl. The latter should not

be stressed too much since the changes are small on a percentage basis, and they were followed in only 3 of the 6 injections; but they appear to be consistent and they make it difficult to maintain the theory that after adrenalin the potassium comes out merely as a result of acid-base changes. Certainly in the case of adrenalin the mobilization of the potassium is independent of the lactic acid.

On the theory that small increases in lactic acid observed might have been the result of the high potassium, we injected KCl in experiments 1 and 2 of table 2 between the two injections of adrenalin. The rise in

TABLE 2

Plasma electrolyte changes after adrenalin

NUMBER	AMOUNT INJECTED	WEIGHT OF CAT	BEFORE ADRENALIN					CHANGE AFTER ADRENALIN				
			K	Lactate	Na	Cl	Cell volume	K	Lactate	Na	Cl	Cell volume
	mgm.	*kgm.*	*m.eq./l.*	*m.eq./l.*	*m.eq./l.*	*m.eq./l.*	*per cent*	*m.eq./l.*	*m.eq./l.*	*m.eq./l.*	*m.eq./l.*	*per cent*
1a	0.04	3.3♂	4.55	2.15	149.3	120.5	32.0	+3.55	+0.27	−0.7	−2.5	+9.0
1b	0.05		3.74	2.14	151.5	118.5	35.2	+5.20	+0.53	−2.1	−1.8	+5.2
2a	0.05	3.4♀	5.48	2.73			40.3	+5.02	+0.11			+2.5
2b	0.04		5.70	3.60	132.1	117.5	34.9	+3.34	+0.26	−3.3	−7.4	+1.0
3a	0.03	2.6♂	5.60	0.67			46.0	+5.35	+0.08			+9.5
3b	0.03		3.77	0.94			51.0	+6.43	−0.07			−1.0

All three cats were anesthetized with dial. Arterial blood samples (5 ml.) were taken in a syringe containing in the needle 0.14 cc. of a solution of 2.1 per cent Na oxalate and 3.9 per cent NH_4 oxalate. Lactic acid was analyzed in whole blood; Na, K and Cl in plasma. Adrenalin was injected in about 20 sec. intravenously in about 1 cc. of 0.85 per cent NaCl. Samples were taken a few minutes before and 1 minute after adrenalin. At least 45 minutes were allowed between the first and second injection. In experiments 1 and 2 an injection of 5 cc. of 0.2 M KCl was given slowly over a period of 1 minute between the two adrenalin injections. The resulting changes in plasma K were typical and are not reported in detail. The lactic acid changes were insignificant.

plasma potassium concentration which was observed was at least as great as that resulting from adrenalin injection but the changes in lactic acid were quite insignificant.

Further details of these experiments are illustrated in figure 3 where the data of experiment 1 (table 2) are plotted.

Muscular activity. It is well known that in muscular activity there is a loss of both potassium and lactic acid (Fenn and Cobb, 1936). This might suggest an escape of potassium as potassium lactate, but this hypothesis is rendered difficult by the finding that the loss of potassium is accompanied by an almost equivalent gain of sodium. These experiments, however, covered a period of about a half-hour so that it is still possible

that the loss of potassium and the gain of sodium do not occur simultaneously. In that case a loss of potassium with lactate would be possible.

To obtain further evidence on this question a series of experiments was undertaken with cat muscle in which the venous blood from the muscles was collected before, during and after stimulation and analyzed for both lactic acid and potassium. The preparation used was similar to that described by Fenn, Wilde, Boak and Koenemann (1939). A strong ligature was passed around the thigh underneath the femoral artery and vein and the sciatic nerve. The venous cannula was inserted into the saphenous vein close to the femoral and pointing toward the heart so that

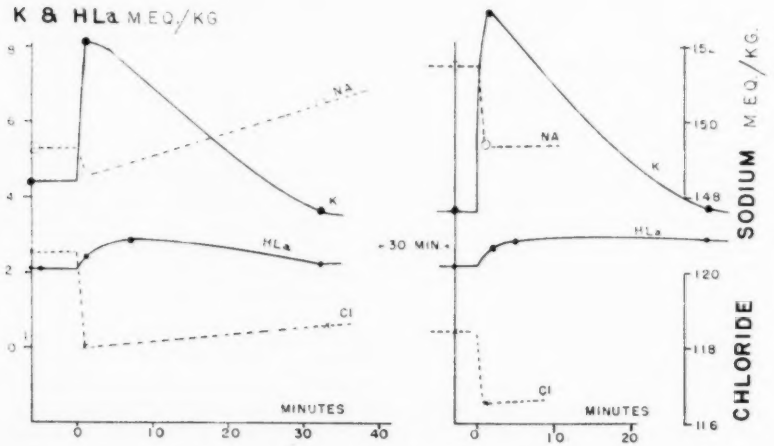


Fig. 3. Effect of two adrenalin injections in a cat. Abscissae, time in minutes after injection. Ordinates: concentrations of K, Na and Cl in plasma and of lactic acid (HLa) in whole blood in milliequivalents per liter. Data from experiment 1, table 2. It is assumed that the concentrations observed before injection did not change until adrenalin was injected.

clamping of the femoral would divert all the blood into the cannula. Clotting was prevented by heparin. When a sample was not needed the blood could be allowed to return to the heart in the usual way by removing the clamp from the femoral vein. The ligature around the thigh gave assurance that all the return blood came through the cannula. Samples of whole blood were used for lactic acid analyses and samples of plasma for potassium.

During the past two years we have done a number of these experiments and have had no difficulty in demonstrating a marked increase in the concentration of potassium in the venous blood which begins apparently

simultaneously with the onset of contraction. Since our experiments were completed a paper by Wood, Collins and Moe (1940) has appeared in which similar experiments were performed on a dog muscle perfused by a heart lung preparation. Our results are similar in general to those reported in the dog with the exception that we have never been able to observe during recovery that the potassium returned to the muscle. In our experiments the potassium level gradually fell in the venous blood to the pre-stimulation level but did not go below that level. Such a return of potassium to the muscle is of course to be expected and our failure to observe it suggests either that recovery is more gradual in the cat than in the dog or that our preparation was not in a sufficiently normal condition to obtain good recovery. It must be admitted that the contractions which we have obtained with this preparation have not seemed to be as well maintained in general as would be expected from our experience with muscles of similar cats in which no attempt to collect the venous outflow had been made. Nevertheless the contractions were initially strong, and there is no reason to suppose that the electrolyte changes, during contraction at least, were not normal.

The changes of potassium and lactic acid in the venous blood for five different experiments are plotted in figure 4. All the blood from a few minutes before the onset of contraction until a few minutes afterwards was collected and analyzed in successive samples. The graphs show that the potassium increases rapidly beginning with the first 10 seconds after stimulation while the lactic acid rises more slowly after an initial lag or even an initial fall slightly below normal. This finding seems to be characteristic of all our experiments. It is striking that the increase in concentration of lactic acid is usually higher than that of the potassium and reaches its maximum only after the potassium level has begun to fall.

These results make it difficult to believe that the potassium comes out into the blood as potassium lactate. The reason for the slower appearance of the lactic acid may, of course, be merely a matter of slower diffusion or of a lower permeability of the membranes to lactic acid. Whatever the reason may be, the fact is nevertheless evident that potassium enters the blood for the most part unaccompanied by lactic acid. It appears likely, therefore, that it enters by exchanging with plasma sodium but the simultaneity of these two processes has not been demonstrated.

Muscular activity in frog perfusions. A further opportunity for studying the potassium loss from muscles in relation to the lactic acid was offered by some experiments in which the hind legs of frogs were perfused through the abdominal aorta. The perfusate was collected by a cannula in the abdominal vein, the renal portal veins being tied off. As a perfusion mixture we followed Saslow (1938) in using a 10 per cent suspension of washed beef red cells in frog Ringer's solution containing 3 per cent acacia.

Further details of this method have been described by Fenn, Koenemann and Sheridan (1940).

If the arterial potassium concentration is high the muscles remove potassium from the blood; if the arterial potassium is low the muscles give up potassium to the blood. This result is a confirmation of the finding of Mond and Netter (1930). The result is illustrated in part by

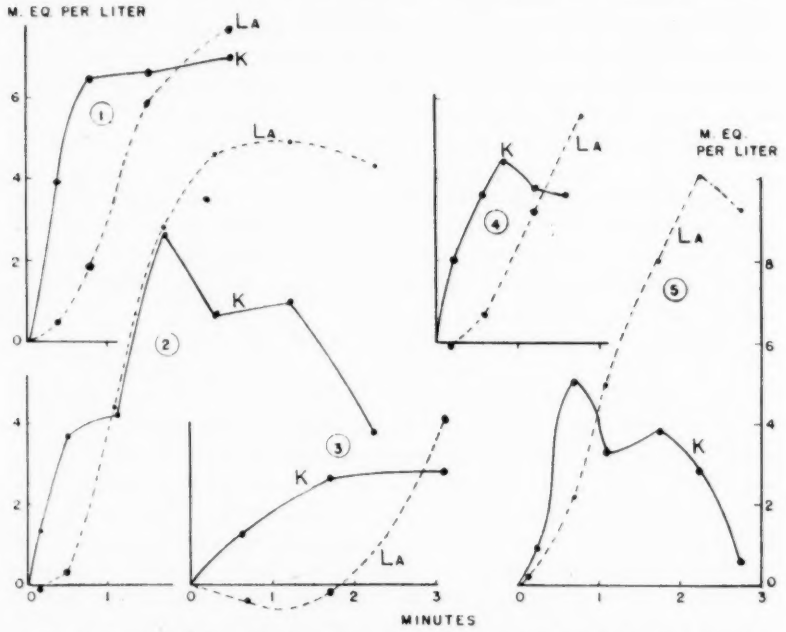


Fig. 4. Graphs of five experiments on cats showing the changes in the potassium and lactic acid concentrations in the venous blood from muscles as a result of electrical stimulation through the sciatic nerve. Dotted lines, lactic acid in whole blood; solid lines, potassium in plasma. Abscissae: time in minutes after the beginning of stimulation. Stimulation in the five experiments: (1) 2.6 min. at 15 per sec., (2) 3 min. at 15 per sec., (3) 4½ min. at 18 per sec.; (4) 3 min. at 42 per min.; (5) 2¼ min. In no. 2 stimulation began 2 min. after the injection of 50 mgm. of iodoacetic acid, and the cat was dead 10 min. later.

figure 5 which represents an experiment in which the potassium content of the arterial blood was suddenly increased for a period and then returned to the original low level. The level of the potassium in the venous blood increases, however, relatively little. Another similar experiment gave an identical result.

The absorption of such large amounts of potassium from the blood afforded an opportunity to investigate the method of uptake of the potas-

sium. For this purpose the pH of the arterial and venous blood was followed both before and after equilibration with 5 per cent CO_2 and 95 per cent oxygen (see fig. 5). In spite of the large amounts of potassium which were being removed from the blood the pH change at 5 per cent CO_2 was negligible. This proves that the potassium did not leave the blood as KOH or in exchange for H ions because titration of the actual perfusion mixture used with known amounts of KOH showed that the buffering capacity was such that the removal of 6.8 m. mols. of K per kgm. from the blood as KOH should have decreased the pH by two units (i.e., from

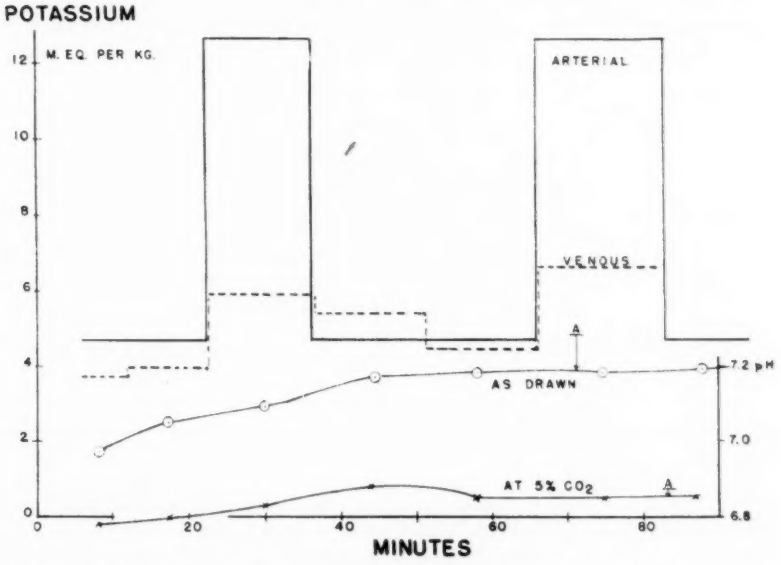


Fig. 5. Effect of varying arterial potassium on the venous potassium concentration in a frog perfusion. The venous pH both as drawn under oil and after being re-equilibrated at 5 per cent CO_2 and 95 per cent O_2 are also plotted. Corresponding arterial pH values are indicated by levels marked *A*.

7.3 to 5.3). The gradual rise of the pH of the venous blood before equilibration (i.e., as drawn) and its approach to the arterial value indicates progressively less acidifying effect due to CO_2 from the tissues and probably means a more rapid flow of blood through smaller fractions of the capillary bed. The total flow decreased from 0.58 cc. per minute at the beginning to 0.15 cc. per minute at the end in spite of an increase of pressure from 21 to 31 cm. of water at 40 minutes on the graph. Increase of K in the arterial blood did not change the arterial pH which was 7.28 in equilibrium with pure oxygen (at either high or low potassium) and 6.87 when equi-

librated with 5 per cent CO_2 and 95 per cent oxygen. These values are indicated on the graphs in figure 5. The slightly lower pH of the venous blood at 5 per cent CO_2 as compared to the arterial probably indicates the addition of lactic acid from the muscles.

Curare. With the same frog perfusion preparation it was possible to test whether the loss of potassium resulting from muscular activity is the result of the contraction or the result of excitation at the neuro-muscular junction. This was done by stimulating after poisoning with curare. After the addition of curare the perfusion was continued until there was no visible response to a single shock. Then the nerve was stimulated with a tetanic current. Analysis of the venous perfusate showed no detect-

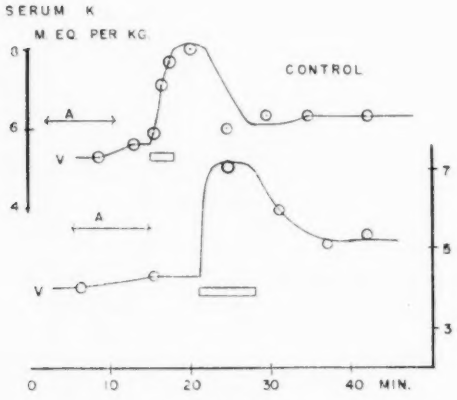


Fig. 6

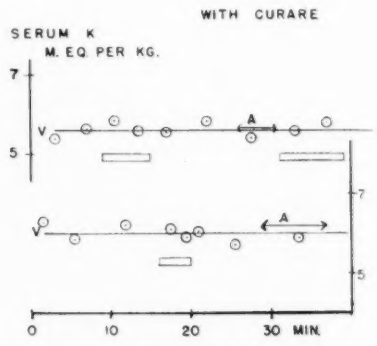


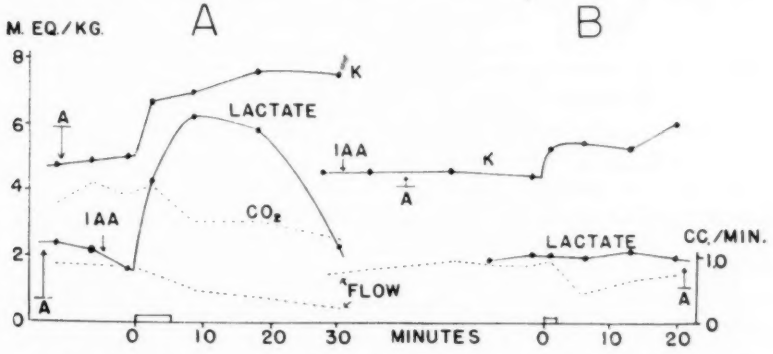
Fig. 7

Figs. 6 and 7. Effects of stimulation on the venous serum potassium content of perfused frog muscles. Figure 6, normal muscles; figure 7, similar muscles poisoned with curare. Blocks indicate periods of stimulation. Points are plotted at the middle of the interval used for the collection of the sample. Corresponding arterial values are indicated by *A*.

able increase of potassium. This fact is illustrated in figure 7, and figure 6 gives 2 control experiments without curare for comparison. Two other unreported experiments with curare gave identical results. Since these experiments were completed, a similar result in the dog has been published by Wood, Collins and Moe (1940). These experiments cast some doubt upon the data of Reginster (1938) showing an increase in the diffusible K after stimulation even when contraction is prevented by curare.

Iodoacetic acid. As a further more crucial test for the independence of potassium and lactic acid in muscular contraction we have studied the liberation of these substances from stimulated perfused frog muscles with and without the addition of iodoacetic acid. The results of two such

experiments are shown in figure 8 A and B. In the former the iodoacetic acid was introduced only 3 minutes before stimulation and did not act for a sufficient time to prevent the formation of lactic acid. Nevertheless the muscles went into rigor after 2 to 3 minutes of stimulation. The potassium in the venous blood remained at a high level, indicating a continued loss from the muscles during rigor. In the experiment of figure 8 B the iodoacetic acid was introduced 30 minutes before stimulation. This resulted in an equally prompt onset of rigor, a smaller contraction, a smaller but continued loss of potassium and no significant increase of lactic acid. Nevertheless this experiment, like others similar to it, indicated that a loss of potassium in contraction can occur without a corresponding loss of lactic acid. It might perhaps be argued, however, that



Figs. 8 A and B. The effects of stimulation of the sciatic nerve of a perfused frog on the lactic acid and potassium in the venous blood, A, 3 min. and B, 30 min. after iodoacetic acid (indicated by arrows). Stimulation periods indicated below by blocks. Arterial levels of K and lactate indicated by A. Abscissae, time from the beginning of stimulation. K in m.eq. per liter of plasma and CO_2 and lactate in m.eq. per liter of whole venous "blood."

the loss of K which is observed is the result of the rigor rather than the result of the contraction per se.

In figure 8 A values of venous CO_2 content are included which show a steady decrease. This would suggest an increased flow but a decrease in flow was observed. Since the arterial perfusate was equilibrated with pure oxygen practically all the CO_2 in the venous perfusate must have been derived from tissue metabolism. It must be concluded, therefore, that the diminished flow indicates a stagnation in these capillaries where the flow was initially least and which therefore contributed most to the CO_2 content of the blood.

A summary of our 15 experiments with iodoacetic acid is obtained from table 3 in which the corresponding changes in potassium and lactic acid are tabulated. For comparison five control experiments without iodo-

acetic acid are included and the experiments with iodoacetic acid are divided into two groups according to the time allowed between the introduction of the poison and the beginning of stimulation. Figures are given in the table for the changes in concentration of lactic acid and potassium which were observed in the first period after stimulation and also the maximum changes which were observed in any later period after stimulation. With only two to twenty minutes for the poison to act there was no appreciable effect but with periods longer than 20 minutes the change in potassium concentration was slightly less than normal, probably due to the smaller contraction, and the change in lactic acid was diminished by an even greater amount. The probable errors of the mean would indicate that the decrease in lactic acid was large enough to be significant. This leads to the conclusion that nearly normal amounts of potassium

TABLE 3

Summary of experiments showing the effect of iodoacetic acid on the average increase of potassium and lactic acid in venous blood due to stimulation

	TIME AFTER IAA	NUMBER OF EXPERIMENTS	Δ K	Δ LACTATE
	min.		*m.eq./l.*	*m.eq./l.*
Control	0	5	a 1.57 ±0.20 b 1.70 ±0.17	a 0.97 ±0.31 b 1.95 ±0.47
IAA	2–17	5	a 1.46 ±0.32 b 2.85 ±0.71	a 2.03 ±0.67 b 4.97 ±1.12
IAA	20–63	10	a 0.81 ±0.15 b 1.14 ±0.14	a 0.10 ±0.05 b 0.18 ±0.06

a = calculated from first period and b = calculated from maximum value in any period following stimulation. Lactic acid is calculated for whole blood and potassium for plasma. The errors given are the probable errors of the means.

may be liberated in muscular contraction without appreciable amounts of lactic acid.

We have also tried stimulating a cat muscle after injection of iodoacetic acid, but have not been able to obtain results differing appreciably from the normal without killing the animal (cf. no. 2, fig. 4). In the course of these attempts we have observed in two experiments (after injection of IAA but without stimulation of muscles) a sudden rise in arterial potassium from 5 to about 10 m.eq. per liter and a subsequent fall toward normal as death approached. In a normal cat there was no corresponding formation of lactic acid but in one cat previously injected with potassium lactate the administration of iodoacetic acid also caused a rise in lactic acid. The meaning of these observations requires further investigation.

Discussion. These experiments have shown that potassium is liberated

into the blood often but not always in company with lactic acid. It could hardly be expected that any one substance could account for all the movements of potassium. All the acids and bases should be considered together. The relative values of the changes in potassium and lactic acid in the different conditions are nevertheless of some interest. In asphyxia and after adrenalin the potassium change is at least twice as great as the change of lactic acid. After hemorrhage the reverse is true. The effect of asphyxia is possibly due to the sympathetic stimulation and liberation of adrenalin which it causes while hemorrhage involves other factors concerned with the loss of blood volume.

In muscular activity the potassium and lactic acid increments are more nearly equal but they have quite different time courses so that they cannot be completely interdependent. The effect of iodoacetic acid helps to dissociate these two substances by decreasing the lactic acid more than the potassium. This evidence is not altogether convincing, however, because it is difficult to be sure that the liberation of potassium in this case is not due to the onset of rigor. It might perhaps be possible to stimulate at such a slow rate that no lactic acid was formed in which case there might still be a perceptible loss of potassium. Although there may still be some connection between potassium and lactic acid insofar as their *liberation* in the muscle fiber is concerned, it is certain that potassium may *appear* in the blood without equivalent amounts of lactic acid.

We have tried various experiments to learn just how potassium enters the blood but so far we are only able to rule out the suggestion that it enters (or leaves) as KOH. The pH at constant CO_2 tension does not change enough to permit this explanation. In two experiments on cats we have tried to settle the question by analyzing arterial and venous blood for Na, Cl, H_2O and HCO_3 in addition to K and lactate before, during and after stimulation. The results remain difficult to interpret, partly because of the small percentage changes in Na and Cl, which are therefore subject to large errors, and partly because of the difficulty or impossibility of knowing for certain just how much venous blood corresponds to how much arterial blood. The conclusions vary according to the basis which is chosen for comparison, whether it be water, solids or red cells, and no one basis is entirely above reproach. In general the water content goes down and the Na, Cl and HCO_3 go up and even on a dry basis they show changes as large or larger than the changes in potassium. It may be, therefore, that the changes in K and lactate are only the most easily detectable but not the most important quantitatively of the acid-base changes which occur in contraction.

SUMMARY

1. In progressive acute hemorrhage in cats the lactic acid increases in the blood more than the potassium (in equivalents) while in acute asphyxia

from clamping the trachea, and after the injection of adrenalin, the increment of potassium is more than twice the increment in lactic acid.

2. In muscular contraction in cats the increments of potassium and lactic acid are more or less equal (or the lactic acid increment is larger), but the rise in lactic acid begins later and reaches its maximum later than does the rise in potassium. In cats the increase in potassium becomes perceptible in a sample of venous blood collected during the first 10 seconds after stimulation begins.

3. In frog perfusions curare completely abolishes the rise in both potassium and lactic acid; iodoacetic acid after acting for a sufficient length of time abolishes or much diminishes the increment of lactic acid due to stimulation without abolishing (although somewhat decreasing) the increment in potassium.

4. Measurements of pH show that potassium does not enter or leave the blood to any extent as KOH. It is also evident that it can enter or leave without equivalent amounts of lactic acid.

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EFFECT OF INSULIN ON NERVE ACTIVITY

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Procedures have previously been employed in this laboratory to determine the action of the convulsant, strychnine, on the nervous system (Heinbecker and Bartley, 1939). More recently the same method has proven successful with regard to the action of two typical anesthetics, ether and nembutal (Heinbecker and Bartley, 1940). Since insulin produces marked modifications in the overt state of the animal attributable to alteration of nervous activity, the same technique has been applied to the study of the action of this substance, the present paper being a presentation of the findings and their interpretation.

The experiments included the investigation of 1, the threshold, accommodation, conduction rate, and absolutely refractory period, of the nerve fiber; 2, the efferent discharge over the phrenic nerve; 3, the afferent discharge over the vagus nerve of sense organs in the lung; 4, several aspects of cortical behavior; in the latter case, among other things, the change in spontaneous activity; the size of the immediate cortical response to stimulation of the saphenous nerve, and of the retina with light; and, also, the insulin effect on the intrinsic periodicity and facilitation in the cortex were studied.

METHOD. Cats were used for the experiments and generally fasted 24 to 72 hours, and in some cases given preliminary doses of 10 units of insulin (Iletin, Lilly) the day before the experiments, to insure that the final doses of insulin administered intravenously at the time of recording would produce a sufficiently marked insulin effect.

In order to immobilize the locally anesthetized animal while recording from the cortex, tetra-methyl-ammonium-iodide was used. About 20 mgm. per kilo were sufficient to produce a curare-like effect, while the drug produced no observable change in the spontaneous cortical records as observed under ether, and did not seem to reduce the size of the cortical response to sensory stimulation as does ether. The drug, however, resulted in a temporary rise of the blood sugar level, and provision for

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its subsequent reduction to definitely hypoglycemic levels had to be made. Figure 1 reproduces two curves to illustrate the time course of the changes in the blood sugar level, the first when the animal was fasted 24 hours but not given insulin on the day of fasting, and the second when insulin was given on the day of fasting. From the curves it is obvious with the dosages of insulin employed, that an hour or more elapses before the blood sugar level drops to the minimum, which even then does not represent a very low value. With longer fasting and repeated insulin injections marked hypoglycemia resulted.

It was in some cases necessary to begin the experiment during deep insulin depression and work back toward the normal by giving intravenous glucose (15 to 20 cc., 5 to 20 per cent in Tyrode's solution). The effects

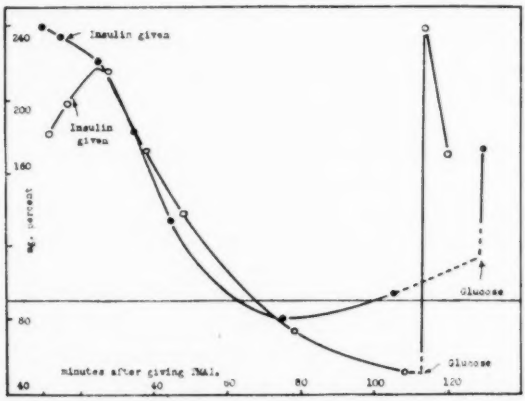

Fig. 1. Herein are two curves showing the blood sugar level following the administration of tetra-methyl-ammonium-iodide. See text for remarks.

of glucose are to be interpreted in light of this. That is to say, if upon its administration the animal was comatose, its effect was to revive it. If the animal was in a convulsive or hyperirritable state, its effect was to quiet it. If given during convulsions, the glucose thus brought the animal back toward the normal state; if given during coma, it also influenced the animal in the same direction, but often into or through the convulsive state.

Results. *The nerve fiber.* To study the effect of insulin on the nerve fiber the animal was first fasted and then given insulin. When the animal was reduced to coma, one saphenous nerve was removed under local anesthesia. In both cases the threshold, accommodation, conduction rate, and absolutely refractory period were determined.

Following the examination of the first nerve, the animal was given glucose, which revived it in a few minutes to the point of becoming spon-

taneously active and very responsive to irritant stimuli. It was then lightly anesthetized with ether and the other saphenous tested in the same manner as the first.

The properties of the first nerve were found to lie within the normal range as indicated by previous studies (Heinbecker, O'Leary and Bishop, 1933). The second nerve was not appreciably different from the first. Figure 2 shows the accommodation curves for both. From the results it can be said that these properties were not modified even when the animal was so deeply depressed that it did not respond to stimuli such as are involved in an operation.

The phrenic discharge. The operative preparation for recording from the phrenic nerve was carried out under local anesthesia and tetra-methyl-ammonium-iodide. The animal was given artificial respiration after cutting the vagi and the efferent discharge over the phrenic studied. To accomplish this the thorax was opened on the right side, the phrenic nerve sectioned above the diaphragm and freed upward for a distance adequate

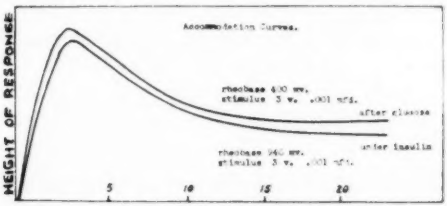

Fig. 2. Accommodation of cat saphenous nerve under deep insulin depression and when revived by glucose.

to permit its placement on the recording electrodes. The lung was kept out of the field by moist packs.

During the excitement state effected by insulin, the amplitude of the discharge was enhanced and prolonged. In addition to this there appeared to be an increase in the discharge frequency of the individual responses in the volley.

Normally, the discharge lasts only the time represented by the usual inspiration (Heinbecker, 1932) and during expiration the nerve is quiet. Our animals were given artificial respiration at a constant rate. The movement of the lungs and chest walls, therefore, set up afferent stimuli which often paced the phrenic discharge. In the border-line convulsive state the discharge, when once set up, sometimes extended for a period comparable to several respiration cycles and thus escaped from its artificial pacing. Not only in this way but in others also synchrony was lost. Under some conditions the phrenic volleys broke up into 3 to 5 bursts during each inspiratory phase of the cycle. At other times the alterna-

tions of bursts and momentary pauses continued through the expiratory phase as well, without regard to the artificial rhythm and forming a uniformly spaced train of rapid volleys at a rate corresponding to that which is seen during panting. Vigorous artificial ventilation with air did not eliminate the panting, but it ceased following ventilation with a mixture of 10 per cent CO_2 in 90 per cent O_2.

The characteristic action of glucose was to reduce the prolonged duration and excessive amplitude of the discharge very markedly, sometimes immediately restoring it to normal proportions. It also eliminated the

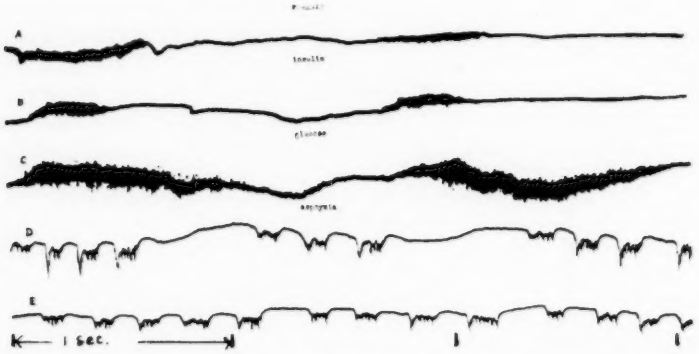

Fig. 3. These records illustrate the kinds of discharge mentioned in the text. A represents the phrenic discharge under a moderate degree of insulinization. When glucose was given with the animal in this state, both the amplitude of the summated discharges in the volley and the duration of the volley were decreased, as shown in B. Line C indicates the effect when artificial respiration is discontinued for about a minute, asphyxia prolonging and increasing the amplitude of the volley. Lines D and E show the phrenic discharge in another animal. In D, the insulinized animal pants during the inspiration phase of the artificial respiration cycle. After the administration of glucose, the panting continues throughout the whole artificial respiration cycle, though the amplitude of the discharges is not so great. This is taken to indicate that insufficient glucose was given to bring the animal all the way back through the hyperirritable stage.

panting rhythm. In the state of coma, however, glucose enhanced the discharge or made it reappear after it had vanished. It should be kept in mind that the enhancement of the response does not follow the administration of glucose to the animal in the normal state, but only when depressed following insulin. (See fig. 3.)

The afferent vagus discharge. Under conditions similar to those used in recording the efferent response over the phrenic nerve, the afferent discharge over the vagus was examined to determine whether during excitement of the central nervous system under insulin administration, the

activity of the peripheral sense organs is also heightened. The nerve was sectioned and freed high in the neck and elevated from the body upon recording electrodes close to the cut end. These electrodes were upon a common carrier provided with a transparent cover, forming a chamber to maintain the nerve in a moist atmosphere (O'Leary, Heinbecker and Bishop, 1934). The lungs are supplied with sensory endings of the vagus nerve which are activated by the expansion of the lungs.

In our experiments, no indication of any increased activity was obtained. From this it is assumed that sense organ responsiveness was not heightened during the convulsive insulin state.

Cortical response—relation of size to stimulus strength. The shape of the response of the sensorimotor cortex changes both in the untreated and in the insulinized animal as the strength of stimulation of a peripheral nerve is increased. In our experiments, the saphenous nerve was sectioned at its distal end and elevated onto the electrodes in the chamber previously referred to. With weak stimuli activating only the A fibers, an early diphasic or triphasic response is evident. With stronger shocks a definite second component, predominantly negative in sign, also appears, and becomes confused with the negative component of the weaker response. This is elicited by the delta fibers in the nerve whose conduction rate is slower. Whereas the onset of the first component followed the beginning of stimulation by about 8 to 15 milliseconds, the peak of the negative wave of the record approaches a value of 45 to 50 milliseconds. With still stronger stimulation, activating the C fibers in the nerve, a much later component also appears. Its latency is about 400 to 500 milliseconds. Due to the way the components overlap it is not easy to detect all increases in size of response. The increase in a given component may appear to diminish one of opposite sign partly concurrent with it (Heinbecker and Bartley, 1940), since the positive phase of the second compensates the negative phase of the first.

In the insulinized animal, the *size* of the immediate response of the sensorimotor cortex to saphenous nerve stimulation continued to increase to a greater degree than normal over the entire range of the saphenous "nerve spectrum." This, of course, did not apply to all states of insulinization, but only to the excitement stage, as judged by other criteria, such as increased muscle tone and lowered threshold for reflex response. It is pertinent to point out that this enhancement of the cortical response was similar to that found under strychninization, but less marked (see fig. 4).

The administration of 5 to 10 cc. of calcium gluconate intravenously greatly reduced and sometimes obliterated this enhancement. This, too, is similar to what happens under strychninization, where calcium is able to prevent or to reduce the severity of convulsions.

Cortical response—flashes of light. The cortical response to a flash of

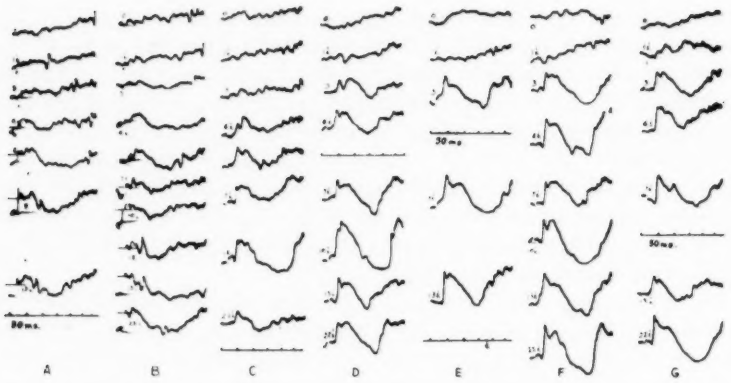

Fig. 4. In this figure the enhancement of the cortical response to electrical stimulation of the saphenous nerve is illustrated by the use of a step-wise series of intensities varying from 1.5 to 22.5 volts, each column of records with the animal in a different state. The stimulus is indicated by a break in the line preceding the response. Column A indicates the outcome with the animal fasted 36 hours, but prior to giving insulin. The threshold stimulus was in the neighborhood of 3 volts, and an increase in the stimulus voltage to 13.5 made little change beyond that of 4.5 volts. In the records the stimulus distortion causes the responses at first glance to be larger than they really are, but closer inspection will reveal their true size and their failure to increase much as stimulus strength is raised. Column B includes records taken 6 minutes after the administration of 10 units of insulin. This period of time made little change from the previous recordings. Column C was recorded 19 minutes after the injection of insulin. By this time the insulin had been able to affect the blood sugar level of the animal. The overt change consisted in hyperirritability and panting. The records show a general increase in the response size, even near threshold, and a greater augmentation of the response as stimulation is made stronger, the effect not stopping at a stimulus strength of 4.5 volts. As was said before, the way in which the components of the response overlap produces a series which does not show a uniform increase in size throughout the range, though the series taken as a whole bears out the conclusion that the components of the responses do increase.

Column D was recorded a couple of minutes after giving about 15 cc. of 15 per cent glucose. The animal had both lost its hypertonicity and ceased panting. Note that the size of the responses has become still greater and the increase over the 4.5 volt level is still more apparent. The glucose evidently changed the animal toward both greater irritability and responsiveness.

Column E was taken about 5 minutes later. At this time the blood vessels had largely recovered from collapse and constriction, typical under insulinization, and which must have lessened the blood supply to the brain and the remainder of the central nervous system and thereby have been responsible in part for some of the symptoms. Twenty minutes after records in column C, column F was recorded, three minutes after a second injection of glucose. The records are even larger than previous ones, and the same increase with stimulus strength still appears. Column G was taken 18 minutes later. The only difference apparent is the dwindling of the relative sizes of responses throughout the whole range from what they had been previously.

light repeated every 4 to 5 seconds was also recorded, with the animal in various stages of insulinization (see fig. 5).

From the consistent results on four animals, it may be said that insulinization can modify the immediate response of the *optic* cortex. It enhances the response during the time the animal exhibits increased muscular tone or is in a border-line convulsive state. Glucose reduces the responsiveness of this cortex promptly, as also does calcium.

Cortical response—paired or repeated stimuli. In the normal animal two stimuli adequate to stimulate only the large A fibers in the saphenous nerve will summate to produce a single cortical response only when they are no more than a few milliseconds apart. Separations greater than about

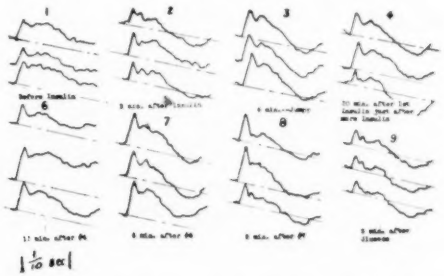

Fig. 5. This figure pictures the response of the optic cortex to retinal stimulation under several conditions: (1) before insulin; (2) 3 minutes after an injection of 8 units of insulin; (3) 6 minutes after the injection. The animal has become hyperirritable and hypertonic, as evidenced by over-response to tapping on the flanks. The cortical response likewise is enhanced; (4) 20 minutes after the first injection and just after a second dose of 4 units: (6, 7, and 8) represent responses during a subsequent period of 27 minutes during which time no further enhancement of response is evident; (9) taken 3 minutes after the injection of 20 cc. of 15 per cent glucose solution, showing a diminution in the size of the response. The onset of stimulation coincides with the beginning of the line.

5 or 6 milliseconds cause the second stimulus to become ineffective (at least under light anesthesia) until the separation is extended to about 30 milliseconds. Beyond this, both shocks are separately responded to, and with further separation the second shock finally elicits a response equal to the first.

After insulin depression has reached a certain point, the stimuli which were originally effective may fail to elicit response when given singly, but may summate when paired or repeated.

In one experiment, in which the animal was fasted and also given insulin the day before, as well as just before recording, the first observation revealed this refractory interval to be 34 milliseconds. Twenty-two minutes later the interval was 80 milliseconds. Fifteen minutes later,

3 cc. of 20 per cent glucose solution was administered intraperitoneally. In 4 minutes the interval had become reduced to 44 milliseconds, and 6 minutes after that the interval had diminished to 31 milliseconds. It is pertinent to note that this same summation phenomenon was observed with animals under strychnine.

Under insulinization the response to the second stimulus never becomes larger than the response to the first. This contrast between the normal and insulinized animal is brought out in figure 6, in which there is a curve for each of the two states. In both cases, it will be noted, there is a first summation period in which the two responses produce a unitary response, larger than either one alone. This is followed by a drop in each curve, representing the onset of the period during which the second stimulus is apparently ineffective. The drop begins later in the insulin curve than

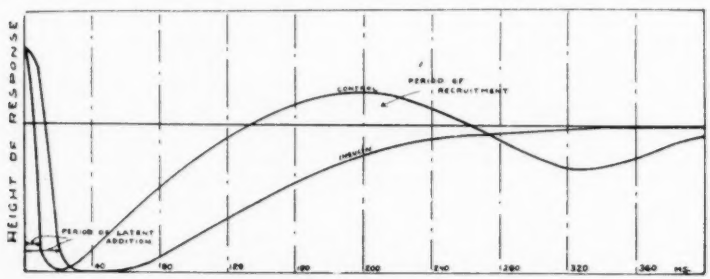

Fig. 6. A diagram showing the relative heights of the response to the second of two stimuli before and after the administration of insulin. Note the periods of latent addition and of recruitment. The latter occurs only under the most favorable conditions. Under insulin it is eliminated, the late period of full effectiveness of the second stimulus is postponed and there is no minimum following the first maximum in the curve.

in the other. After a while both curves rise again. The insulin curve rises later and slopes off to a plateau at about the level of the horizontal line marking the height of a response to a single shock. The other curve rises earlier and extends above the line, indicating a period during which facilitation may occur when the individual shocks of repeated stimulation are separated by intervals represented in the diagram. This phenomenon was pointed out recently by Heinbecker and Bartley (1940). With separations greater than indicated for the maximum second response, a period of diminution sets in, to be replaced still later by a second maximum.

Rhythmicity in the size of the cortical response was first pointed out by Bishop (1933). This phenomenon was brought out very clearly by Bartley (1936) in studying the response of the optic cortex to electric shocks applied to the optic nerve. Most of his animals were under very light ether anesthesia. When dial anesthesia was used the result was similar,

but the period of the excitability rhythm was longer, corresponding to the slowing of alpha rhythm under dial.

Spontaneous cortical activity. Not only are there definite changes in the cortical response to peripheral stimulation, but there are also alterations in spontaneous cortical activity, observable under various degrees of insulinization and following the use of substances which reverse the symptoms. The observations made here are of similar type to those made of the spontaneous activity when the animal was placed under the influence of strychnine, ether, or nembutal.

The effects on both specific and spontaneous responses may be summarized as follows: Glucose and calcium, each may reduce the size of the specific cortical response. If the animal is comatose, glucose restores it to general responsiveness, and restores the much diminished or vanished specific response we are measuring. Calcium does not have this latter effect. It only reduces the response in a hyper-responsive animal (Heinbecker and Bartley, 1939). Calcium increases the frequency of the alpha rhythm in the insulinized animal and diminishes the amplitude of the waves involved when large. Glucose and calcium increase the frequency of the alpha rhythm above the rate found under insulin, and tend to restore toward normal the amplitude of the waves exaggerated by insulin, the original slowing having been similar to that found by Hoagland, Rubin and Cameron (1937).

Comparison of cortical and sub-cortical responses. In animals so deeply comatose from insulin that the spontaneous waves of the cortical record had disappeared, electrodes placed in the basal ganglia of the opposite side still recorded marked activity. In some cases the animal had progressed so far that giving glucose did not to any degree restore the waves. In others, a slight restoration was possible. The retention of a recordable active state in the basal ganglia after the cortex is totally depressed and beyond restoration is not at all surprising, for animals are generally viable when the cortex for any reason fails to give a record. Nevertheless, we have herein a demonstration of one of the differential effects of insulinization.

Discussion. As was already pointed out, our results show that the effect of the hypoglycemic state resulting from insulin administration on the excitation and response processes of the central nervous system of the cat are similar to those already described for strychnine administered intravenously (Heinbecker and Bartley, 1940). The maximum excitatory effects obtainable following insulin action are less pronounced and less prolonged than those possible after strychninization. This follows reasonably from the fact that insulin administration must soon deplete the cells of the central nervous system of the carbohydrate necessary for their function (Holmes and Holmes, 1925). The results, showing that the intravenous administration of glucose will restore to normal within a

few minutes markedly depressed function in the central nervous system, strongly support the contention of other investigators (Himwich and Nahum, 1932) that glucose is the essential material for nerve cell activity. Recovery from a similar degree of depression produced by strychnine is much slower and cannot be effected by glucose administration. Apparently strychnine thwarts the chemical reactions necessary for nerve cell function, whereas hypoglycemia deprives them of essential material without being toxic. Clinical experience indicates, however, that the acidosis associated with prolonged hypoglycemia is also toxic in that recovery from it following glucose administration is a relatively slow process.

The exciting effects of hypoglycemia as well as those of strychninization modify activity in the vasomotor centers in a manner to bring about both arterial and venous constriction in the body generally. We have observed that this results in both ischemia and asphyxia. In the depression period following the excitation the peripheral constriction of arteries and veins is descreased. Cardiac dilatation, especially of the right heart, is evidence of a coincidental weakening of the myocardium. This perpetuates and intensifies the asphyxia with its resulting depression of all cellular activity.

Experiments have been carried out in this laboratory to determine the effects of carbon dioxide and of anoxemia on the function of the peripheral nerves (Heinbecker and Bishop, 1928) and of the central nervous system (Bishop, 1930, unpublished data by the authors). The effect of carbon dioxide (10 per cent in 90 per cent oxygen) is to produce excitatory effects similar to those produced early in hypoglycemia and by mild strychnimization. The effect of anoxemia (inhalation of pure nitrogen) is essentially depressant with sometimes a brief early period of excitation if the anoxemia is effected rapidly. According to theory that may be the result of an accumulation of acid metabolites rather than a direct effect of the deprivation of oxygen. These facts are referred to because it is felt that the effects of hypoglycemia and of intravenous strychninization which manifest themselves in the intact animal under our experimental conditions are not simple but represent the end-result of an integration of many factors.

The work in this laboratory has dealt, from time to time, with several aspects of cortical response; namely, its spontaneous features including the alpha rhythm, the effectiveness of the second of two supraliminal peripheral stimuli, and of repeated stimulation, the summation of two sub-liminal peripheral stimuli, and the effect of drugs on these phenomena.

It has become progressively clearer that in such experimentation we are dealing with two groups of cells, one expressed in the immediate specific cortical response to peripheral stimulation, and the other expressed in the alpha rhythm. The second group is spontaneously active and the first is not. Normally the two groups react on each other. A specific response tends to set in motion an alpha train, while the height of a specific response to an isolated stimulus is partially dependent upon where in the alpha

cycle it falls. Strychnine topically applied differentially affects the two, the activity of the spontaneous elements disappearing while certain components of the specific response still may be enhanced. Under strychnine the specific response, though itself enhanced, may no longer set off an alpha train. This is not due simply to synaptic blocking between the response and spontaneous groups but also to an actual depression of the alpha elements inasmuch as alpha waves no longer appear spontaneously. Any repetitiousness that local strychnine produces is manifest in the activity of the non-spontaneous, non-rhythmic elements responsible for the immediate specific response. The effectiveness of repeated stimulation is dependent not only upon the place in the alpha rhythm (spontaneous excitability cycle) each stimulus falls, but also on the spacing of the stimulation and the amount of repetition. This still holds even in the absence of discernible spontaneous alpha waves. Possibly this is indicative of a close similarity in the time course of the excitability processes of the spontaneously and non-spontaneously active elements. Any substance, so far tested, which modifies the rate of the alpha rhythm, whether it is to slow it or accelerate it, produces a like effect on the period which must elapse before a second of two stimuli will produce a measurable immediate cortical response.

A duality of cell groups such as we have evidence for in the cortex, exists in the median nerve cord of the Limulus heart (Heinbecker, 1936). In it there are large pacemaker cells which are spontaneously and rhythmically active. There are also smaller cells which are not spontaneously active but are activated by the pacemaker cells. The integrated outcome of the activity of the pacemaker cells is a slow heart beat of about 20 per minute, each cell being active only a small part of this cycle. The response represents the integration of a progressively spreading activity in a chain of elements. The result of extrinsic excitation of this system depends upon the phase of the cycle at which the stimulus is delivered. A strong stimulus when properly placed elicits a response consisting in the activity of those large and small cells which have recovered from their just previous activity and in the setting up of a new rhythm in definite relation to the stimulus, the old one generally having disappeared. This prototype, inasmuch as the evidence gained from it is more direct, helps to justify our interpretation of the phenomena of the cortex and to clarify what is undoubtedly a more complex situation.

A correlation between the changes effected by hypoglycemia on the elements of the nervous system and the reactions of the body as a whole in this state is of interest. A basis for such excitatory effects as hyperirritability, sweating, trembling and convulsions has been afforded. The mental confusion of depression associated with the convulsive seizure doubtless is an expression of early cortical depression at a time when the lower brain centers and the spinal cord are still hyper-reactive.

SUMMARY

Responses of various parts of the nervous system under insulin were compared with those under standard conditions approaching the normal as nearly as possible, and with the results under strychnine.

The *excitatory* effects following insulin administration are evidenced by a lowering of the threshold for the immediate cortical response to stimulation of the saphenous nerve and an increase in its amplitude and duration. This is associated with an intensification and prolongation of the discharge in certain peripheral axons as is illustrated in the efferent discharge over the phrenic nerve.

Peripheral sense-cells evidently do not participate in the insulin excitatory effect, for no enhancement of the afferent discharge of the vagus nerve was obtained in the hyperexcitable state of the animal. The same lack of excitation is true of nerve fibers.

Depressant action of insulinization is first exhibited in the slowing of nervous processes and later in their diminution and failure. Depression is exhibited in the prolongation of the summation interval (interval of latent addition) and the recovery period of the cortex following peripheral nerve stimulation; by reduction of the cortical response; and by the obliteration of facilitation as measured in our experiments. Depression is seen also in the slowing of the alpha rhythm.

The susceptibility of the different parts of the nervous system to the action of hypoglycemia varies greatly. The cortex may be depressed to extinction at a time when the basal ganglia and the medullary centers still show well integrated activity. Furthermore, the basal ganglia are excited to a greater degree than is the cortex itself. The peripheral synapses, peripheral sense organs and axons are unaffected even just prior to the death of the animal.

The general concepts which have been arrived at through extended work on the nervous system, particularly the cerebral cortex, in this laboratory were discussed in order to orient the findings on insulin of this particular study.

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THE RATE OF GLYCOGENOLYSIS IN THE ISOLATED LIVERS OF SEVERAL SPECIES OF LABORATORY ANIMALS

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It is generally believed that the rate of breakdown of glycogen in the excised liver is extremely rapid. Most investigators have emphasized the necessity for the rapid destruction of glycogenase in the determination of liver glycogen or in its preparation from this source (Bodansky and Fay, 1931). On the other hand even more rapid glycogenolysis may occur in the excised brain tissue. Kerr and Ghantus (1937) found that 80 to 85 per cent of the glycogen is lost from this source within 15 minutes of post-mortem autolysis. In fact Kerr (1938) states that "the interval between excision from the living animal and fixation in alkali should not exceed 10 seconds since glycogenolysis proceeds rapidly (Kerr and Ghantus, 1937)." The glycogen in the abalone muscle on the other hand is extremely resistant to hydrolysis *in vitro* as demonstrated by Petree and Alsberg (1929) who were able to prepare large amounts from the tissues of these animals after long periods of storage. We have repeatedly confirmed these observations in this laboratory.

Cori, Cori and Schmidt (1939) have noted that the diastase content of the blood of rabbits is low compared with such other laboratory animals as the dog, rat and guinea pig. In the perfused liver of the rabbit the rate of glycogenolysis was found to be very slow unless phosphate was added. In the latter case a maximum of 70 per cent disappeared in one hour whereas in control tests the rates varied between 4 and 12 per cent in a similar interval.

In the present investigation, a study has been made of the rate of disappearance of glycogen from the livers of well-fed rats, pigeons, guinea pigs, rabbits, and a dog when this organ was excised and kept at a temperature of 37°C. for various periods of time.

METHODS. In most cases the animals were given large amounts of glucose approximately 12 hours before being sacrificed. Amytal was used as an anesthetic. The liver was divided into several portions, which were weighed in tared centrifuge tubes, and 40 per cent KOH was added immediately to one sample.

The other tubes were kept in an incubator at 37°C. for appropriate periods after which the glycogen remaining was determined. In all cases glycogen was estimated by the procedure of Good, Kramer and Somogyi (1933).

RESULTS. The most satisfactory method for comparison of the rate of glycogenolysis in the various species studied seems to be on the basis

TABLE 1

The liver glycogen in per cent (columns I) and the per cent decrease (columns II) after standing at 37° for various periods after excision from animal

TIME OF INCUBATION	PIGEON		RABBIT		DOG		GUINEA PIG		RAT	
	I	II	I	II	I	II	I	II	I	II
hours										
1	9.51	0	7.51	12.9	4.10	10.5	6.63	16.9	3.18	31.2
1C	9.46		8.63		4.58		7.98		4.62	
	(1)		(3)		(1)		(2)		(6)	
3	7.18	15.0	6.75	21.8	3.60	21.4	3.92	21.3	2.10	55.8
3C	8.45		8.63		4.58		4.98		4.75	
	(2)		(3)		(1)		(2)		(11)	
6	6.92	24.0	6.05	29.9	3.13	31.7	3.71	36.9	1.52	73.6
6C	9.11		8.63		4.58		5.88		5.75	
	(2)		(3)		(1)		(2)		(8)	
12	7.58	36.4	4.33	49.8	1.58	65.5	2.62	62.7	0.84	84.2
12C	11.92		8.63		4.58		7.02		5.33	
	(2)		(3)		(1)		(2)		(7)	
24	6.76	44.7	3.09	65.5	0.62	86.5	1.12	82.5	0.59	89.7
24C	12.22		8.95		4.58		6.40		5.72	
	(2)		(4)		(1)		(4)		(7)	
48	5.14	43.2	3.32	63.7						
48C	9.05		9.14							
	(3)		(2)							
72	2.01	26.1	3.55	64.2						
72C	2.72		9.91							
	(1)		(1)							
96			3.32	66.5						
96C			9.91							
			(1)							

Periods marked C represent the level of glycogen before incubation for the group concerned.

Figures in parentheses represent the number of animals used in each test. There are the same number of control as experimental values in each case.

of the percentage decrease from the original level. This value is calculated as follows: (Control per cent − Per cent after incubation)/Control per cent.

The average per cent decrease of liver glycogen at the various periods investigated as well as the absolute glycogen values are summarized in table 1.

Glycogenolysis in excised liver tissue apparently is not as rapid in any case as is generally supposed. After one hour an average of 31 per cent had disappeared in the rat liver, while only 10 to 16 per cent was lost in the case of the dog, rabbit and guinea pig. No appreciable change was found with the excised pigeon liver at this period. After 12 hours the liver glycogen had reached a low value with the rat liver, although appreciable quantities still remained with the other species. In fact about 75 per cent of glycogen was still found after 72 hours in the pigeon liver and 34 per cent after 96 hours in the case of the rabbit liver.

Although the control levels are considerably higher in most cases in the experiments on pigeons and rabbits, the slower rate of breakdown of glycogen can not be ascribed to that fact. For example, in the one hour period no demonstrable decrease was noted in the case of the pigeon, while the average drop in liver glycogen with the rats was 1.44 per cent. The corresponding lowering for the dog, guinea pig and rabbits was 0.48, 1.35 and 1.12 per cent respectively. Even after the 6 hour incubation period, similar discrepancies were to be noted. Thus the decrease amounted to 2.19, 2.58, 2.17 and 1.45 per cent respectively for the pigeon, rabbit, guinea pig and dog while the corresponding value for the rat was 4.23 per cent. Moreover, an inspection of the individual experiments indicates no definite correlation between the height of the glycogen level at the start of the experiment and the extent of breakdown. After 6 hours the decrease in amount of glycogen in the livers of rats does not exceed that of the other species because it is approaching a blank value. Thus it appears that the rate of glycogenolysis is most rapid in the liver of the rat followed in order by that of the dog, guinea pig, rabbit and pigeon.

SUMMARY

The rate of glycogenolysis in excised liver tissue kept at 37°C. has been found to be much slower than generally believed. The most rapid rate obtains with the rat followed in order by the dog, guinea pig, rabbit and pigeon. In the last case nearly 75 per cent of the original was still found after an incubation period of 72 hours.

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A STUDY OF THE NERVE-FREE SMOOTH MUSCLE OF THE AMNION OF THE CHICK

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The direct effect of physical and chemical agents on muscle tissue can be unequivocally studied by using naturally occurring nerve-free muscle tissue. A number of sources of naturally occurring nerve-free muscle are available. That found in the blood vessels of the human placenta and umbilical cord has been used; but at term such vessels are senile and the premature placenta is not readily available. The aneural embryonic heart has also been used, but graphic records of its activity cannot be made. That found in the amnion of the chick is perhaps most readily available and can be prepared so as to make graphic records.

The structure of the amnion musculature of the chick has been studied rather extensively, more recently by Verzar (1), Pierce (2) and Lewis (3). The absence of nerves has been confirmed by many (1–6). Although the function of the musculature is unknown, it has been suggested (7) that it agitates the amniotic fluid and gently rocks the embryo. *In situ* in the hen's egg, 5 to 14 days after incubation, the amnion musculature has been observed to contract rhythmically at a rate of from 16 to 20 per minute (1), and apparently in the form of waves (2). Epinephrine inhibits the muscle in situ, according to Langley (8). According to Baur (6), isolated strips of the amnion of the goose and chick respond to drugs. This work was undertaken to investigate further some of the physiological and pharmacological properties of this nerve-free smooth muscle and to repeat some of Baur's observations.

EXPERIMENTAL. *Examination for nerves.* Although nervous tissue has never been found in the amnion, a search was made by the methylene-blue technique (9), using the tissue from embryos incubated for from 8 to 16 days. Twenty-four specimens were examined, using a 0.15 per cent solution of the dye in normal saline and incubation at 37°C. A portion of the specimen was examined at periods up to 45 minutes. No nervous tissue was found.

In the remainder of this work, the specimen was always dissected in an isotonic bath at 39°C., using the solution of Van Dyke and Hastings (14) or of Sollmann and Rademaekers (12).

Method of recording contractions. The amnion of a chick from a small breed, as used in this work, is a rather delicate structure. In order to record contractions, a lever consisting of a very light paper straw was used. To reduce friction a loop of silk thread served as a fulcrum. Prior to the 10th day the structure was found to be too delicate to produce good graphic records; after the 14th day rhythmicity disappeared. So the specimens used in this study ranged from the 10th to 13th day of incubation, the fertilization time being known.

Solutions used in the bath. It was first thought advisable to use a solution having the chemical composition of the amniotic fluid of the chicken. It was found that the composition, specific gravity, and pH of the amniotic fluid varies considerably after the 9th day of incubation (10, 11). The composition of amniotic fluid, then, is not identical with blood plasma, and, since the salts in bird's blood range from 0.9 to 0.95 per cent, mammalian saline solutions were used in the bath.

Several saline solutions were used in a preliminary study to determine which was the most suitable: Locke's and Tyrode's with their various modifications (12), Sollmann and Rademaekers' (12), Alexander and Hastings' (13), and Van Dyke and Hastings' (14). The Alexander-Hastings solution depressed and abolished rhythmicity (15), and was not used. We did not try a tissue culture medium (3). The Van Dyke-Hastings solution hereafter called D-H solution, was the most satisfactory for the preservation of rhythmicity, it being aerated with 5 per cent CO_2 and 95 per cent oxygen. The Sollmann-Rademaekers solution, hereafter called S-R solution, was also satisfactory, it being aerated with air by the usual method. The pH of the solutions was shown to be constant throughout the duration of the experiments. The pH of the S-R solution was 7.8, of the D-H solution, 7.4. The volume of fluid in the bath was 240 cc. and the isolated strip was suspended deeply so that the composition of the fluid could be altered without mechanically disturbing the strip.

The effect of temperature. The optimal range of temperature for rhythmicity was found to lie between 38° and 42°C. Six preparations were studied to determine more carefully the optimum temperature. In these preparations it was found to be 41°C., which is approximately the temperature of a bird. The bath was maintained at 41°C. in all subsequent experiments. A higher temperature increased the rate of the rhythm, but it was not sustained. At a lower temperature the rhythm decreased and then disappeared.

Rhythmicity and tonus. Practically every preparation studied showed some rhythmicity and tonus which persisted for about 20 minutes; it rarely persisted longer than 60 minutes. Some preparations manifested only a feeble rhythm; yet, they would respond well to drugs. In some preparations the rhythmicity was continuous; in others it was intermittent

or occurred in periods. This latter phenomenon had to be considered in the interpretation of the response of the strip to drugs and ions.

The various types of rhythmicity and the variations in rate and tonus are illustrated in the tracings. Tracings *1* and *2* in figure 1 illustrate the more frequently observed rhythm and tonus changes.

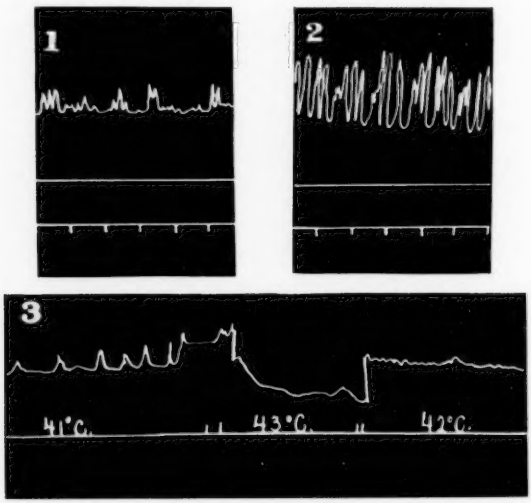

Fig. 1. Tracings *1* and *2* illustrate the variations in rhythmicity and tonus changes observed in the amnion muscle. Tracing *3* illustrates the loss of tone or the relaxation that occurs when the muscle is warmed to 43°C., or illustrates heat paralysis with recovery.

TABLE 1

Showing the rate of rhythmic contractions of the amnion of the chick

DAY	AVERAGE* FIVE-MINUTE INTERVAL	RANGE FIVE-MINUTE INTERVAL	AVERAGE RATE PER MINUTE
10th	34.5	21-60	6.9
11th	34.8	9-53	7.0
12th	16.7	4-36	3.3
13th	20.1	10-31	4.0

* Six specimens for each day.

Incidental observations indicated that the younger specimens were more active. To examine this phenomenon further six strips each were prepared from embryos on the 10th to 13th days of incubation. The results are shown in table 1. The amplitude of the rhythm varied considerably from specimen to specimen, and because of the difficulty of accurately recording

and comparably preparing the strips, the measurements of amplitude were not considered to be worthy of comparison.

When innervated smooth muscle manifesting tone is warmed to 43°C., it elongates (16, 17). When the amnion of the chick was warmed to 43°C., rhythmic contractions disappeared and the muscle elongated. On lowering the temperature to 42°C., the rhythmic contractions returned (tracing *3*, fig. 1). On warming the amnion strip to 46–47°C., the muscle lost its irritability permanently (17). Pierce (2) noted that on cooling the amnion of the chick to 25°C. the tonus of the muscle disappeared and the muscle appeared to be flabby. This observation was readily confirmed; but after the muscle had been cooled to and kept at 22 to 23°C. for five minutes, it would, after a short quiescent period, manifest rhythmic contractions and tonus changes on being placed in the bath at 41°C.

Response to stretch. It could not be demonstrated that a change in tension *per se* influenced rhythmicity and tonus. The mechanical difficulties may have interfered with this experiment. It is possible that by using some more sensitive method of observation a slight response may be detected, although we doubt it. The amnion muscle of the chick is certainly not as sensitive to stretch as the innervated smooth muscle of the intestine and gall bladder (18).

The muscle *in situ* responds to pinching and a wave of contractions sometimes apparently spreads from the point of local contraction.

The response to changes in calcium. Using D-H solution, which contains phosphates, it was found that on increasing the calcium ion from 1.06 m.eq. per liter to 1.54, the tone and rate of contractions were augmented in 4 of 5 specimens. Other experiments were performed with S-R solution, which contains no phosphates. Using the S-R solution, it was found that when the calcium concentration was raised from 2.16 to 2.84 m.eq. per liter, no significant change occurred. Increasing the Ca concentration from 2.16 to 3.02 m.eq. per liter very definitely increased the activity of the muscle. Increasing the Ca concentration from 2.16 to 4.58 m.eq. caused a marked increase, seven experiments being performed. In three other experiments the Ca concentration was raised from 1.62 m.eq. per liter to 2.70; an increase in the activity of the muscle occurred in each instance. Lowering the Ca concentration through the same range decreased the activity of the muscle. These results are similar to those of others working with smooth muscle (15).

The response to changes in potassium. Using S-R solution, the concentration of potassium was raised from the original of 5.1 m.eq. per liter to 5.93 and to 6.375. This change increased the rhythmic contractions, which also occurred when the potassium concentration was raised to 10.05. It was observed that when the initial potassium concentration was 5.1 m.eq. per liter and then the concentration was reduced to 3.18 m.eq. per

liter, the muscle ceased contracting spontaneously and did not recover. Six specimens were used. The changes in concentration were effected as in the case of the Ca studies, by removing an aliquot of the original solution from the bath and then adding the requisite amount of the same type of solution having a higher or lower concentration of potassium so that the concentration of the potassium in the bath would be increased or decreased to the desired extent.

These effects of potassium on the muscle of the amnion are similar to the effects of potassium on the smooth muscle of the uterus (14).

The response to changes in magnesium. The addition of magnesium to the S-R solution, which contains no magnesium, decreased the rate of the rhythmic contractions, especially when the magnesium ion concentration was raised to 2.04 m.eq.; the first definite decrease in rhythm occurred when the magnesium concentration reached 1.04 m.eq. per liter. When the strip was placed initially in D-H solution, which contains 1.0 mM. of magnesium per liter, the magnesium concentration had to be raised to 3.04 m.eq. per liter before the decrease in rhythm was definite. Only three experiments were performed because it is generally agreed that magnesium depresses the activity, but prolongs the rhythmicity of smooth muscle (12, 14).

The response to changes in phosphate. Four specimens were studied in D-H solution in which the phosphate concentration was varied by the addition of either $NaH_2PO_4H_2O$ or Na_2HPO_4. These were added so as to increase the phosphate ion concentration from the initial level up to 3.0 and 4.08 m.eq. per liter. The addition of the acid phosphate increased the activity of the muscle whereas the addition of basic phosphate did not produce a definite change.

The response to drugs. The drugs used were added to the bath which contained 240 cc. either of S-R or D-H solution. The simple addition of 12 mgm. of sodium chloride had no effect on any of the preparations. No drug was added in sufficient quantity to change significantly the pH of the solution. The threshold dose was used to designate that amount of drug which evoked a response in the majority of preparations used. The strip was washed between each application of a drug.

Morphine sulphate. Eleven strips were treated with from 4 to 12 mgm. of morphine sulphate. The threshold dose was 8 mgm. or 1:30,000 concentration. A decisive increase in the rate of the rhythm, in amplitude and a contraction or increase in tone occurred after 12 mgm. or a concentration of 1:20,000. An illustration of a case in which the rate of the rhythm and the level of tone was increased is shown in tracing *1*, figure 2. In no instance was relaxation or inhibition observed.

Baur (6) occasionally observed stimulation with morphine, but reported relaxation to be the predominating response. It is to be noted that a relatively large concentration of morphine is required to produce contraction.

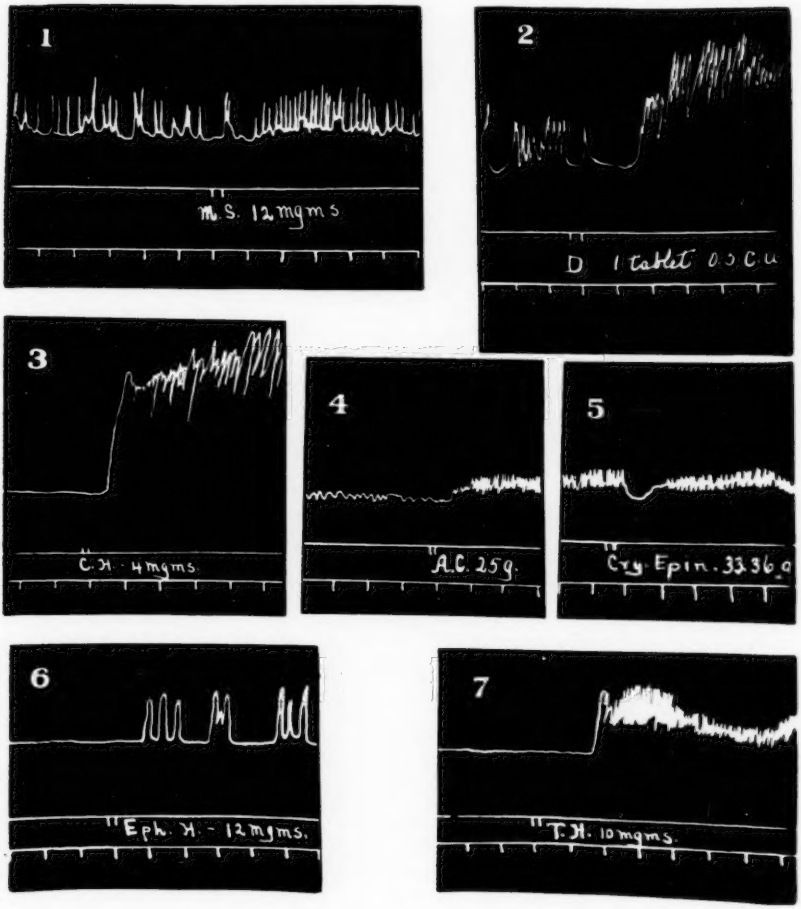

Fig. 2. Tracing *1* illustrates the type of response that occurred to a concentration of 1:20,000 or 1:30,000 of morphine sulphate.

Tracing *2* illustrates the type of response that occurred to 1 or 2 cat units of digalen per liter of solution.

Tracing *3* shows a marked contraction due to cocaine in a concentration of 1:60,000, which is not followed by paralysis if the muscle is promptly washed.

Tracing *4* illustrates increased activity induced by 25 gammas of acetylcholine bromide, or 104 gammas of the bromide or 74.8 gammas of the base per liter of solution.

Tracing *5* shows inhibition caused by 33.36 gammas of crystalline adrenalin, or 139 gammas per liter of solution.

Tracing *6* shows the response of a muscle that had lost its rhythmicity to ephedrine in a concentration of 1:20,000.

Tracing *7* shows the effect of tyramine in a concentration of 1:24,000.

Digalen. This preparation of the water soluble principle of digitalis (19) caused the muscle to contract. The threshold dose was 1.0 cat unit per liter of solution in five experiments. The response of the muscle to approximately 2 cat units per liter of solution is shown in tracing *2*, figure 2.

Cocaine hydrochloride. In 14 experiments it was found that a concentration of 1:480,000 of this drug constituted a dose sufficient to increase the rate and amplitude of the rhythmic contractions in most cases. A concentration of 1:240,000 was required to cause a contraction or an increase in the tonus level. Tracing *3*, figure 2, shows the response of a strip to a concentration of 1:60,000. Larger doses caused paralysis, unless the preparation was immediately washed.

Baur (6) observed only paralysis in response to cocaine, but he performed only two experiments.

Acetylcholine bromide. In 10 experiments the minimum effective dose required to increase the activity of the muscle was 26 gammas (concentration of 1:38,400,000) of the drug per liter of solution. This is equivalent to 18.7 gammas of acetylcholine per liter. The rate of the rhythm and usually the tonus level and amplitude of contractions were increased by that dose. Tracing *4*, figure 2, shows the response to 25 gammas of the bromide per liter of solution.

Acetylcholine bromide and atropine sulphate. The effect of atropine sulphate alone was studied in 10 preparations. The results were variable. A concentration of 1:480,000 of atropine sulphate appeared to stimulate slightly the muscle. The rhythmicity of some strips was inhibited by a concentration of 1:48,000; however, in one instance a concentration of 1:24,000 was required to depress activity and other strips were not depressed even by this concentration.

Using 10 strips it was found that a concentration of 1:480,000 of atropine sulphate abolished the activity induced by 1:120,000 of acetylcholine. After atropine in a concentration of 1:480,000, a concentration of acetylcholine of 1:240,000 was ineffective in causing a contraction. After a concentration of 1:48,000 of atropine, a concentration of 1:48,000 of acetylcholine bromide was ineffective (fig. 3).

It was clear that large doses of atropine were required to inhibit the movements of the strip and even then some were refractory. Much smaller doses antagonized the effect of acetylcholine bromide. No attempt was made to determine the quantitative relations of the atropine-acetylcholine antagonism (32).

Adrenalin. Crystalline adrenalin was employed to determine the threshold dose. In 20 experiments, in which concentrations in the bath of from 1:76,800,000 to 1:120,000 were used, the dose required to temporarily inhibit the muscle was a concentration of 1:9,600,000 or 104 gammas per liter of solution. The response of a strip when 33.36 gammas were

added to the bath is shown in tracing 5, figure 2. With the threshold dose of 104 gammas per liter the inhibition was definite, but of short duration. Chloretone which is added to adrenalin as marketed, also inhibited the muscle (2); but when diluted to the extent that obtains when commercial adrenalin is usually employed, it had no effect. Hence, the threshold dose of commercial adrenalin on the amnion musculature was found to be the same as that for the crystalline product.

Ephedrine hydrochloride. In 16 experiments, concentrations of this drug were used which varied from 1:240,000 to 1:12,000. The threshold concentration was 1:48,000. It caused an increase in rate, tonus and amplitude. In tracing 6, figure 2, is shown the response of a muscle to 12 mgm. of the drug, a concentration of 1:20,000. It is to be noted that the muscle was not showing spontaneous contractions at the time the drug was applied.

Fig. 3. This tracing shows abolition of rhythmicity and tonus by atropine sulphate in a concentration of 1:48,000, added at *a*. The addition of acetylcholine at *b* failed to cause a significant effect.

Tyramine hydrochloride. In 9 experiments, concentrations of this drug were used which varied from 1:480,000 to 1:24,000. The dose required to increase the rate, amplitude and tonus was 1:80,000. The effect of the drug in a concentration of 1:24,000 is shown in tracing 7, figure 2.

The effects of cocaine hydrochloride on the action of adrenalin, ephedrine hydrochloride and tyramine hydrochloride. In this group of experiments adrenalin, ephedrine or tyramine was added to determine the responsiveness of the strip. The solution was changed and cocaine hydrochloride was added. Then the original drug was added in the same amount as used prior to the addition of cocaine. Crystalline adrenalin was added in a concentration of 1:240,000 to 1:1,200,000 before and after the addition of cocaine hydrochloride in a concentration of 1:48,000. In every instance adrenalin inhibited the response to cocaine, but the inhibition was less in duration than before the addition of cocaine. No evidence of cocaine potentiation of adrenalin inhibition was obtained. Ephedrine hydrochloride (1:24,000 to 1:48,000) increased the activity before the addition of cocaine, but had no effect after the addition of cocaine. The same was true of tyramine (1:40,000).

In 4 other determinations ephedrine hydrochloride in concentrations of 1:48,000 to 1:24,000 was used both before and after 1:80,000 to 1:48,000 concentrations of cocaine hydrochloride. The ephedrine hydrochloride increased the activity of the muscle before, but had little or no effect after the cocaine was added. Likewise, in 4 determinations with tyramine and tyramine hydrochloride in concentrations of 1:80,000 to 1:40,000 both before and after cocaine hydrochloride in concentration of 1:48,000, the tyramine increased the activity of the muscle before, but had little or no effect after the cocaine.

Thus, after cocaine, adrenalin had its characteristic inhibitory reaction on the amnion muscle. Cocaine did not augment or reverse the effect of adrenalin on the muscle. Cocaine does not reverse the effect of ephedrine and tyramine on amnion muscle. To determine whether cocaine acts synergistically with ephedrine and tyramine smaller concentrations of the drugs will have to be used, since the concentration of the cocaine used may have caused a maximal contraction.

Discussion. The physiological properties of the nerve-free smooth muscle of the amnion, as far as it has been examined, appear to be very similar to those of innervated smooth muscle. One qualitative difference appears to exist, namely, this nerve-free smooth muscle appears not to be stimulated by stretch. In this respect it is analogous to the retractor penis of the dog, which through innervated has no nerve plexus, and does not respond to stretch. To our knowledge no smooth muscle free of a nerve plexus has been shown to be stimulated by stretch (20).

It is frequently stated that such drugs as adrenalin and acetylcholine mimic the action of autonomic nerves by stimulating their nerve endings. Our observations and those of Langley (8) and Baur (6) on the amnion muscle show that this is not necessarily true. Neither is it true for the nerve-free blood vessels of the human placenta (21) nor for the aneural embryonic heart of Fundulus (22) or of the chick (23, 24). Nerve-free smooth muscle may relax (amnion) or may contract (placenta) in response to adrenalin, and may contract (amnion, always) or may relax slightly or contract markedly (placenta) in response to acetylcholine. The response to acetylcholine is antagonized by atropine (amnion, placenta, heart) and augmented by eserine (placenta). The response to adrenalin is abolished by ergotoxine (placenta). Thus these *neuromimetric agents or "chemical transmitters" act directly on non-innervated smooth and cardiac muscle cells.*

The question of the effect of the ingrowth of nerves on the amnion muscle and on the response of the musculature of the blood vessels of the placenta and intestine to drugs can perhaps never be answered. Evidence regarding the question is available for the *heart of Fundulus* (22). Before nerves grow into the heart of this fish, which occurs after it has an otherwise adult

morphology, acetylcholine has a "contracture-like" effect (decrease in diastolic size) which is antagonized by atropine. After the nerves enter the heart, acetylcholine inhibits the heart (in diastole). In this animal the ingrowth of nerves apparently *reverses* and also *sensitizes* the response of the heart to acetylcholine. In the *chick heart*, the ingrowth of nerves apparently does not cause a reversal of the effect of either adrenalin or acetylcholine, but the results with different concentrations of the drugs are so variable as not to permit a conclusion regarding sensitization.

These considerations, along with the greater sensitiveness to these chemicals which apparently occurs after innervation and which would develop a more definite direction to the response and facilitate intercellular conduction (27), would, as Euler (21) has pointed out, harmonize the divergent conceptions of Elliott (26) and Langley (8), regarding the developmental physiology of the autonomic nervous system.

Are excitatory and inhibitory "receptors" for adrenalin and also for acetylcholine inherently present in the same cell? If so, it must be assumed that the adrenalin-inhibitory receptor predominates in the amnion and the adrenalin-excitatory receptor predominates in the placenta. In the case of the heart of Fundulus, the ingrowth of nerves may either sensitize an acetylcholine-inhibitory receptor inherently and latently present, or produce a new receptor, or reverse the one inherently present. Each possibility must be considered and may occur in the adult, in view of the effect of progestin on the functional innervation of the uterus of the cat (27). A developmental pharmacological study of the apparently doubly innervated melanophores of certain fishes would be of interest in this connection, since one nerve contracts and another expands the melanophore (25).

It is clear that atropine, which *per se* has no marked effect on the contractile state of non-innervated cells, antagonizes and prevents the effect of acetylcholine. This shows that atropine opposes the action of acetylcholine by acting on the cell (28, 29) and either the cell must have an atropine receptor, according to Clark (28), or there exists a chemical antagonism between the two drugs, a possibility indicated by Henderson and Roepke (29). That atropine does not paralyze the contractile substance of the non-innervated cell is shown by the fact that cocaine causes a contraction of the atropinized amnion. It therefore may be supposed (21) that atropine in opposing acetylcholine acts on the "fixing power" or *membrane permeability* of the amnion muscle cell.

Cocaine dilates the placental vessels but contracts the amnion muscle. Either the contractile substance or the "receptor substance" differs in the two muscles. The question of cocaine-adrenalin synergism is more involved. One is tempted to conclude that cocaine sensitizes the excitatory but not the inhibitory responses of non-innervated smooth muscle to

adrenalin. But it is known that cocaine sensitizes the innervated bronchial muscle to the inhibitory effect of adrenalin, but not intestinal and urogenital muscle. If the foregoing generalization is made, it is necessary to assume that the ingrowth of nerves into bronchial muscle influences the muscle cell so that the inhibitory effect of adrenalin is potentiated by cocaine. But it is unnecessary to make the additional assumption that in this instance cocaine acts on the nerve ending rather than on the cell, since the ingrowth of nerves may modify the direction or the intensity of the permeability change in the cell membrane induced by cocaine, and we know that cocaine can cause non-innervated smooth muscle to contract or to relax.

SUMMARY

1. The 10th to 14th day amnion of the chick, which is nerve-free, exhibits rhythmicity and tonus changes in a bath of isotonic saline solution. The optimal temperature is about 41°C. At 43°C. it loses rhythmicity and tone but recovers both at 42°C. It survives at 22 to 23°C. for a short period, but loses its vitality at 46 to 47°C. In the saline solutions used rhythmicity rarely persists longer than one hour.

2. The ionic environment influences the motility of this muscle in a manner similar to that observed for innervated smooth muscle.

3. The amnion muscle does not appear to be influenced by stretch. It responds locally to a mechanical and electrical stimulus, and occasionally a wave of contraction appears to be propagated from the point stimulated.

4. The muscle is inhibited by adrenalin (1:9,600,000) and contracts in response to acetylcholine (1:38,000,000) the action of which is antagonized by atropine. Thus these neuromimetic agents act directly on this nerve-free smooth muscle as well as on that of the blood vessels of the placenta (24). The available evidence indicates that the ingrowth of nerves may sensitize and reverse the reaction of smooth muscle to these agents.

5. Atropine in concentrations which antagonize acetylcholine has little or no effect on the amnion muscle; in such doses it appears to stimulate slightly.

6. Adrenalin opposes the contraction of the amnion muscle caused by cocaine, ephedrine and tyramine. Ephedrine and tyramine have little or no effect after cocaine.

7. Morphine sulphate (1:30,000) and digalen (1.0 cat u. per liter) cause the amnion muscle to contract.

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THE RÔLE OF INSULIN IN CARBOHYDRATE METABOLISM

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Two actions are ascribed to insulin in carbohydrate metabolism: that of increasing glucose oxidation and that of increasing glycogen deposition, particularly in muscle. Although such effects are possible, and in some circumstances have been proven to take place, they cannot be used as a basis for an adequate explanation of all the known facts concerning insulin, diabetes, and carbohydrate metabolism. The general literature in this field today stresses particularly the action of insulin in increasing oxidation of glucose, without giving much importance to the increased carbohydrate storing action of this agent. It was our purpose in the work to be presented in this paper to study the relative importance of this second function of insulin in carbohydrate metabolism.

Our first experiments were carried out on depancreatized dogs. The general aim of the work was to determine the relative amounts of insulin needed during the assimilation of carbohydrate and during the post absorptive periods. This latter requirement has been called the "basal insulin requirement" (1). The first dog (table 1) had high carbohydrate feeding days interspersed between fasting days so that the animal practically maintained a steady weight. On the feeding days the attempt was made to give the insulin that was needed for the food. The animal was fed every two hours from 8 a.m. to 8 p.m. and given insulin with each feeding. In the first 3 feeding days, despite administration of 65, 73, 75 units respectively the dog excreted appreciable amounts of sugar in the urine indicating that the insulin was not given in excess. On the 3 first fasting days the dog was given 5, 6 and 5 units insulin respectively and showed urine sugar only on the third day. For the remaining days the urine was kept sugar-free with large doses of insulin on feeding days and small on fasting days. Although the urine showed no sugar on the feeding days the insulin administered could not have been excessive since the dog never had any hypoglycemic attacks. That the dog was kept in a steady state as regards nutrition is indicated by its weight. Furthermore the caloric value of the food given on each feeding day was over double

the daily metabolic requirement that might be expected from the animal's weight. The energy supply of the animal on the fasting days must have come very largely from the sugar and protein fed on the previous day. Since absorption would be practically complete 4 hours after the last feeding we may consider that for 16 hours the animal was absorbing food which was partly utilized then but was mostly put away for use during the succeeding 32 hours. Certainly more of the food would be actually burned during the latter period than during the former, yet by far the bulk of the insulin was needed during the feeding period. The insulin required during the fasting period is no more than the basal need (1).

The point may be raised however that this small amount of insulin needed on the fasting days was necessary for the ultimate combustion of the carbohydrate taken on the feeding days and stored for later use.

TABLE 1

DATE	SUGAR	MEAT	INSULIN	URINE SUGAR	MORNING BODY WEIGHT
	gms.	*gms.*	*units*	*gms.*	*kgm.*
12/ 2/39	500	200	65	4.7	16.7
12/ 3/39	0	0	5	0	
12/ 4/39	700	250	73	14.0	
12/ 5/39	0	0	6	0	17.0
12/ 6/39	0	0	5	13.0	
12/ 7/39	600	550	75	21.0	
12/ 8/39	0	0	6	0	
12/ 9/39	500	100	55	0	17.0
12/10/39	0	0	5	0	
12/11/39	600	200	65	0	16.5
12/12/39	0	0	6	0	16.8
12/13/39	600	300	65	0	16.4
12/14/39	0	0	6	0	

The observations made on the next animal bear on this point. This depancreatized dog (weight 15 K) was given a meal on the first day consisting of 245 grams sucrose and 300 grams meat. (This was the food needed daily to keep the animal in weight balance.) Blood sugar determinations were carried out thereafter at frequent intervals to serve as a guide for the insulin that was required to keep the blood sugar normal. The attempt was made to give just that amount of insulin that was necessary for this.

The results are given in table 2. It is apparent that the animal needed extra insulin from the time of feeding up until about 3 p.m. Thereafter an hourly dose of 0.5 unit insulin sufficed to maintain a steady state. (The duration of action of the larger previous doses is not more than 3 hours (2).) On the next day the animal was fasted and the hourly insulin

requirement needed to maintain a constant normal blood sugar level was found to be 0.5 unit per hour. The same value was obtained on the day following this which was also a fast day. It is reasonable to assume that the source of energy for the period from 3 p.m. to 9 p.m. was the food (predominantly carbohydrate) taken previously. This feeding however was just enough for one day's needs so that by the second day of fasting the animal must have been depending mostly on body fat and a small amount of body protein as a source of energy. Yet the insulin needs are the same for the two periods. That is, the dog required no extra insulin for the extra carbohydrate being metabolized from 3 p.m. to 9 p.m. on the first day. From this it appears unlikely that basal insulin is needed

TABLE 2

TIME (FEBRUARY 8, 1939)	BLOOD SUGAR	INSULIN
		units
8:10 a.m.		4
9:40	Food mixture	
10:30		2
11:15	168	
11:50		2
12:25 p.m.	103	
1:25	114	2
2:25	95	2
3:25	80	1
4:25	75	0.5
5:25	87	0.5
6:25	83	0.5
7:25	87	0.5
8:29	89	0.5
9:25	91	0.5

for oxidation of carbohydrate, but merely to prevent excessive amounts of body protein being broken down to form new glucose (3).

We carried out similar experiments on the depancreatized rat—an animal that does not require basal insulin. The scheme of the experiment was to determine to what extent this preparation could store fed carbohydrate with and without insulin. The high carbohydrate diet given consisted of glucose 68 per cent, Osborne and Mendel salt mixture 4 per cent, commercial casein 18 per cent and "Galen B" vitamin B complex 10 per cent. The vitamin B complex contained 8.6 per cent protein and 59 per cent carbohydrate. The rats had water ad lib at all times. The normal rat is capable of storing fed carbohydrate rapidly since he will maintain steady weight on a regime of a day each of alternate fast and feeding of this diet, as shown in table 3.

The rats were depancreatized by an adaptation of the method of Shapiro and Pincus (4) and the animals were selected for the experiments only if they excreted 8 grams or more sugar per day in the urine on the high carbohydrate diet given above. The animals so selected were subjected successively to 3 regimes, viz: 1, the high carbohydrate diet allowed at all times; 2, alternate days of fast and feeding of high carbohydrate diet; 3, alternate days of fast and feeding plus insulin. The insulin (plain) was administered in 3 doses of 3 units each given at 8 a.m., 2 p.m. and 10 p.m. This amount of insulin was not enough to take care of all the sugar eaten

TABLE 3

Weight response of normal rats to a regime of alternate fasting and feeding with high carbohydrate diet

ANIMAL	NUMBER OF DAYS ON REGIME	WEIGHT AT BEGINNING	WEIGHT AT END
		grams	*grams*
1	10	110	120
2	10	148	159
3	10	104	103
4	10	132	136

TABLE 4

ANIMAL	CONSTANT FEEDING			ALTERNATE FEEDING AND FASTING			ALTERNATE FASTING AND FEEDING PLUS INSULIN		
	Number of days	Weight at first	Weight at end	Number of days	Weight at first	Weight at end	Number of days	Weight at first	Weight at end
A	7	192	198	12	198	170	12	210	204
B	11	152	150	5*	162	122	12	150	150
C	6	170	186	6	172	166	6	186	190
D	8	136	132	8	190	122	8	154	123
E	6	136	140	6	140	101	6	160	140
F	6	153	150	6	164	118	6	194	186
Averages		156	159		171	133		176	165

* Animal was so weak at end of third fasting period that test had to be terminated.

since the animals always excreted considerable quantities of glucose in the urine. We refrained from giving larger doses from fear of producing hypoglycemia. The results are given in table 4.

The order in which the different animals were subjected to these regimes was varied, in some cases they started on 3 and some on 2. If an animal lost weight on any regime he was given a period of daily feeding plus insulin in order to bring him back to normal before starting the next experimental period. This fact together with the natural growth of the animal during the whole experiment accounts for the differences in the weight of a given animal at the start of the different test periods in some cases.

It is apparent from these results that the tissues of the depancreatized rat can derive adequate nourishment from a high carbohydrate diet and can be kept in a steady state if the animal has access to food at all times. It does not need insulin to utilize sugar. However it has difficulty in storing this food stuff as is indicated by the fact that it cannot maintain its weight if subjected to alternate feeding and fasting periods. It does

TABLE 5

	1	2	3
Constant feeding			
Weight at beginning of period	155	152	138
Weight at end of period	153	154	136
No. of days in period	8	9	9
Alternate fasting and feeding			
Weight at beginning of period	153	127	130
Weight at end of period	132	106	105
No. of days in period	6	4*	4*
Average food eaten, grams per day	29	28	33
Average urine glucose per food day, grams.	16.6	11.8	17.7
Average urine nitrogen per food day, grams	0.57	0.675	0.69
Average urine glucose per fast day, grams	0.8	0.6	0.5
Average urine nitrogen per fast day, grams	0.19	0.350	0.20
Alternate fasting and feeding plus insulin			
Weight at beginning of period	132	106	136
Weight at end of period	136	114	132
No. of days in period	6	8	6
Average food eaten, grams per day	26	28	27
Average urine glucose per food day, grams.	5.5	4.2	4.7
Average urine nitrogen per food day, grams	0.32	.30	.38
Average urine glucose per fast day, grams	0.9	0.8	1.1
Average urine nitrogen per fast day, grams	0.19	0.19	0.16

* Rats became so weak on third fasting day that regime had to be discontinued.

much better if insulin is given with the feeding which makes it possible for the animal to store carbohydrate to be used in the fasting period.

Three diabetic rats were carried through a series of experiments similar to those already recorded but with additional observations on food eaten, and urinary nitrogen and glucose. The order of the periods for the first two rats was 1, feeding regime; 2, alternate fasting and feeding; 3, alternate fasting and feeding plus insulin. For the third rat 1, feeding regime; 2, alternate fasting and feeding plus insulin; 3, alternate fasting and feeding.

The results are given in table 5. They show the same weight changes as noted in the previous rat series. In the alternate feeding and fasting regime the rats rapidly lose weight if not given insulin with their food. On constant feeding they stay even. They do not eat more when given insulin but rather, somewhat less. The giving of insulin results in a marked reduction of excretion of sugar in the urine, and this "saved" sugar is stored in the body to serve as fuel to carry the rat through the fasting period. The urine nitrogen figures suggest that insulin effected some retention of food protein on the feeding days. This would help to some degree to maintain body weight. However insulin produces no essential difference between the urine nitrogens of the fasting days of the two regimes which indicates that this protein retention did not contribute to energy needs on the fasting day. Although some of the loss of weight on alternate fasting and feeding without insulin was undoubtedly water lost along with tissue loss, it is unlikely that there was loss of weight from dehydration due to glycosuria. The animals had access to water always. Furthermore the high sustained glycosuria of the constant feeding periods resulted in no loss of weight indicating that this did not cause dehydration.

Discussion. Our results indicate that the main disturbance in diabetes is not due to inability to oxidize adequate carbohydrate. The tissues of the diabetic animal are capable of utilizing glucose (5, 6, 7). Our findings support the view that much of the trouble in diabetes due to insulin lack results from the inability of the animal to store carbohydrate. It has been abundantly proven that insulin may increase the storage of carbohydrate as body glycogen. Cori and Cori (8) have studied the effect of insulin on glucose disposal in rats. In this animal under the conditions they used, a large part of the disappearing glucose is deposited as muscle glycogen. Their experiments were of comparatively short duration (4 hrs.) so that the amounts of sugar involved were quite small in comparison with those that we were concerned with in our work. A simple calculation will show that the stored carbohydrate in our rats cannot all be in the form of glycogen. Cori and Cori (8) report that a rat metabolized some 14.5 calories per 100 grams body weight in a day and this would require about 3.5 grams carbohydrate. This amount could in no way be stored as muscle and liver glycogen. Our dog results indicate the same thing. De Nayer (9) working in Boukaert's laboratory used rabbits to study insulin effect. He administered just that amount of glucose that was needed to keep the blood sugars normal. He found that insulin under these conditions disposed of large amounts of glucose, but that there was no increase in muscle glycogen. The glucose must have gone into some other form. Boukaert (10) has suggested that it is

transformed into fat and that one of the important actions of insulin is to increase the conversion of glucose to fat. As pointed out above, the large amounts of carbohydrate that the insulin aids in storing can be accounted for as glycogen only to a small degree. Most of it must be in the form of fat. If insulin has this effect when the body is actually increasing its content of fat it would probably have the same action when the body is in equilibrium as regards fat. This would mean that when the animal is absorbing just enough carbohydrate to supply its metabolic needs, the action of insulin is to convert some of this carbohydrate to fat; at the same time an equivalent amount of fat would be burned.

Such a concept would require a more general transformation of glucose to fat than has been assumed in the present conventional notion of metabolism. According to this teaching it is only when there is a large excess of carbohydrate in the food over that needed for current metabolic needs that it is changed to fat. Such assumptions have resulted from the belief that one has conversion of carbohydrate to fat only when the R.Q. is greater than 1. But the R.Q. of the entire animal gives little indication of the intermediary processes that may be taking place in the different tissues of the animal (11). An R.Q. of less than 1 in an animal on a high carbohydrate diet does not exclude the possibility of the transformation of a part of the carbohydrate of the diet to fat. Such a process could be taking place in some special tissue (i.e., the liver) and if the other tissues of the body were burning an equivalent amount of fat at the same time the resultant R.Q. of the two processes would be the same as that of the direct burning of the carbohydrate. The recent interesting findings of Schoenheimer and his co-workers (12) using deuterium to tag compounds, give support to this concept. They found that in mice that were given heavy water to drink and were on a carbohydrate diet, the fatty acids of the body were replaced after six days by new fatty acids containing deuterium. They also fed mice, in which the depot fat was marked with deuterium, on a bread diet and found the concentration of tagged fat became steadily less, indicating constant new formation of fat. Barrett, Best and Rideout (13) obtained similar results under these conditions and found the amount of fat in the liver was increased but the concentration of tagged fat here was markedly decreased. We must believe then that there is a much more general conversion of carbohydrate to fat in the normal organism than was formerly supposed. Our findings suggest that this conversion is decreased in diabetes, and that insulin administration increases it. We do not maintain that this is the only action of insulin. It certainly may play an important rôle in the metabolism of other foodstuffs. Thus in some species it is essential for the economy of body protein (3) and for the proper catabolism of fat (14).

SUMMARY

The important action of insulin in carbohydrate metabolism is in bringing about the storage of sugar at the time it is absorbed.

When large amounts of carbohydrate are absorbed by the diabetic organism, a large part of this foodstuff that insulin aids in disposing of must be changed to fat.

Evidence is presented supporting the view that an important action of insulin is to accelerate the transformation of glucose to fat. Without insulin this conversion is either greatly retarded or does not take place at all.

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